UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS: PSIQUIATRIA

TESE DE DOUTORADO

MEDIADORES INFLAMATÓRIOS NO TRANSTORNO BIPOLAR

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Tese apresentada ao Programa de Pós-Graduação em Ciências Médicas: Psiquiatria da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Doutor em Psiquiatria.

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SUMÁRIO

LISTA DE ABREVIATURAS

ADNF	*activity-dependent neurotrophic factor*
AP-1	*activator protein 1*
BCL2	*B-cell CLL/lymphoma 2*
BDNF	*brain derived neurotrophic factor*
BH4	tetra-hidrobiopterina
c-JNK	*c-Jun N-terminal kinases*
cIAP	*cellular inhibitors of apoptosis*
CREB	*camp response element binding*
DA	dopamina
DTI	*diffusion tensor imaging*
ECA-NIMH	Estudo da Área de Captação Epidemiológica
FADD	*Fas-associated death domain*
FLIP	*FLICE-like inhibitory protein*
GSK3beta	*glycogen synthase kinase-3 beta*
IKB	*inhibitor of KappaB*
IL-1	interleucina-1
IL-1R	receptor de interleucina-2
IL-2	interleucina-2
IL-3	interleucina-3
IL-4	interleucina-4
IL-5	interleucina-5
IL-6	interleucina-6

IL-6R	*interleukin-6 receptor*
IL-7	interleucina-7
IL-8	interleucina-8
IL-9	interleucina-9
IL-10	interleucina-10
IDO	indolamina 2,3 dioxigenase
INF-gama	interferon-gama
IRA-K	*interleukin-1 receptor-associated kinase*
IRF-1	fator regulatório do INF
JNK	*jun N-terminal kinases*
KA	ácido quinureico
KYN	quinurenina
L-DOPA	L-3,4-dihidroxifenilalanina
LPS	lipopolissacarídio
MYD88	*myeloid differentiation primary response gene* (88)
NFκB	*nuclear factor kappa B*
NGF	*nerve growth factor*
NO	óxido nítrico
QUIN	ácido quinolínico
SNC	sistema nervoso central
SOD	superóxido dismutase
STAT1a	ativador de transcrição 1a
STAT-3	*signal transducer and activator of transcription 3* (duas subunidades)
TAB1	*transforming growth factor-activated protein kinase 1-binding protein 1*

TB	transtorno bipolar
Th1	T *helper* 1
TNF-alfa	fator de necrose tumoral-alfa
TOLLIP	*toll interacting protein*
TRADD	TNF *receptor associated death domain*
TRAF2	*TNF receptor-associated factor 2*
TRAF6	*TNF receptor associated factor 6*
TRP	triptofano
WCST	*Wisconsin Card Sorting Test*
5-HIAA	ácido 5-hidróxi-indolacético

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RESUMO

Objetivos: Comparar os níveis séricos de citocinas entre voluntários saudáveis e em pacientes bipolares tipo 1 em mania, depressão e eutimia. Comparar os níveis séricos de quimiocinas entre voluntários saudáveis e pacientes bipolares tipo 1 em eutimia. Metodologia: Sessenta e um pacientes bipolares foram recrutados para a avaliação dos níveis séricos de citocinas. Desses, 24 estavam eutímicos, e 23 e 24 estavam em mania e depressão, respectivamente. Este grupo foi comparado a outro, composto por 25 voluntários saudáveis. As seguintes citocinas foram exainadas através de citometria de fluxo: TNF-alfa, IL-2, IL-4, IL-6, IL-10, IFN-gama e comparadas entre os grupos. Além dessa análise, 30 pacientes bipolares tipo 1 foram comparados a igual número de voluntários saudáveis para estudar os níveis séricos das seguintes quimiocinas: CCL2, CCL3, CCL8, CCL 9, CCL10, CCL11 e CCL24, dosadas através de ELISA *sandwich*. Resultados: Duarnte a mania, as citocinas pró-inflamatórias IL-2, IL-4 e IL-6 estavam elevadas nos pacientes com TB em comparação com o grupo de voluntários saudáveis. Já os pacientes em episódio depressivo, apresentavam somente elevação da IL-6. Não houve modificação dos níveis de citocinas nos pacientes eutímicos, exceto pelo aumento da IL-4. Quanto as quimiocinas, enquanto os níveis séricos de CXCL10 estavam elevados nos pacientes bipolares, os níveis de CCL24 estavam diminuídos.

ABSTRACT

Objectives: Compare serum levels of cytokines between healthy volunteers and patients with bipolar disorder type 1 in mania, depression and euthymia. Compare serum levels of chemokines between healthy volunteers and bipolar patients in euthymia.

Methods: Sixty-one bipolar patients were recruited for assessment of serum cytokine levels. Of these, 14 were in euthymic state, 23 and 24 were in manic and depressive episodes, respectively. A healthy comparison group included 25 healthy volunteers. Cytokines involved in Th1/Th2 balance, such as TNF-α, IL-2, IL-4, IL-6, IL-10, IFN-γ, were examined by flow cytometry. In addition, serum chemokine levels of 30 euthymic patients with BD type I and 30 healthy volunteers were investigated and compared. The chemokines assessed were CCL2, CCL3, CCL8, CCL 9, CCL10, CCL11, and CCL24.

Results: During mania, proinflammatory cytokines, IL-2, IL-4 and IL-6, were increased in comparison with healthy subjects. Patients in depressive episode showed only increased IL-6 levels. There were no significant differences in cytokine levels between patients in remission and healthy subjects, except for IL-4. Regarding chemokines, patients with BD showed significant differences in chemokine levels when compared with healthy subjects. While serum levels of CXCL10 were increased, CCL24 levels were lower in bipolar patients when compared with controls. There was no statistical difference in the serum levels of CCL2, CCL3, CCL24, CXCL9, and CXCL11 between patients and controls.

1 INTRODUÇÃO

A última década testemunhou uma mudança radical na visão do papel do cérebro em relação ao sistema imunológico. Se até o século XX se acreditava que o cérebro era uma região relativamente isolada do sistema imunológico, protegida pela barreira hematoencefálica, hoje se considera que o sistema nervoso central (SNC) e o sistema imune se influenciam mutuamente (1, 2, 3). Parte dessa mudança de paradigma deriva das descobertas das diferentes funções da glia, que revelaram que a micróglia se constitui em um elemento capaz de responder a estímulos imunológicos variados, inclusive da periferia e dos astrócitos (4). Além disso, essas células são capazes de comunicar-se com os neurônios usando moléculas neurotrasmissoras e neuromoduladoras (5). Paralelamente, demonstrou-se que, na íntima relação entre o sistema imune e o SNC, uma desregulação da resposta imunológica pode fazer parte da fisiopatogenia de diversas doenças neuropsiquiátricas, como esquizofrenia (6), doença de Alzheimer (7), depressão maior (8,9) e transtorno obsessivo-compulsivo (10, 11, 12, 13, 14,15,16).

Os primeiros dados a respeito do papel da inflamação nos transtornos de humor derivam de modelos animais de depressão maior (17) e de estudos em humanos com essa patologia (18). Em roedores, a administração sistêmica de mediadores inflamatórios, como a citocina interleucina-1 (IL-1) e o fator de necrose tumoral-alfa (TNF-alfa) induz comportamentos semelhantes a sintomas depressivos (19, 20). De maneira similar, em humanos, a administração terapêutica de interferon-gama (INF-gama) pode induzir depressão pela ativação da resposta inflamatória no SNC e na interferência desses mediadores com o metabolismo de monoaminas, especialmente

serotonina (21,22). Existe um robusto corpo de evidência corroborando a associação entre inflamação e depressão maior (22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35,36,37,38). Vários genes associados a suscetibilidade para depressão codificam mediadores inflamatórios (26, 39) que interagem com fatores ambientais (como trauma e maus-tratos na infância), modulando a vulnerabilidade para depressão (40, 41, 42). Adicionalmente, desequilíbrios em certas citocinas recentemente foram associados a características específicas da depressão maior, como o comportamento suicida (43), e foram usados como preditores de resposta ao tratamento (44). No transtorno bipolar (TB), os achados são menos consistentes, mas, ainda assim, potencialmente relevantes. Esta tese tem como objetivo explorar a relação entre o sistema imune e o cérebro no que diz respeito ao TB, inserindo-se, assim, em um novo campo de conhecimento, a psiconeuroimunologia.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 Características clínicas do transtorno bipolar

O TB é um transtorno mental complexo, multifatorial, potencialmente grave, com episódios recorrentes e que está associado com elevada morbidade clínica e psiquiátrica (45, 46). O Estudo da Área de Captação Epidemiológica (ECA-NIMH) (47), conduzido nos Estados Unidos a partir de 1980, mostrou uma prevalência de 0,8% do TB tipo 1. Vários estudos posteriores mostram uma prevalência ao longo da vida de 0,5% a 7,5%, dependendo da amostra e da amplitude dos critérios diagnósticos (48,49,50). Além da alta prevalência, como o TB tende a iniciar com uma idade média de aproximadamente 20 anos (51,52), ele acaba afetando o indivíduo na sua fase mais produtiva da vida, tornando-se um importante gerador de incapacitação. De fato, segundo dados da Organização Mundial da Saude, o TB é considerado uma das dez principais causas de incapacitação no mundo (53).

A despeito do avanço no tratamento, este não apresenta eficácia completa para todos os pacientes. Alguns deles experimentarão episódios frequentes, estados mistos (sintomas maníacos e depressivos concomitantemente), complicações relacionadas a abuso de substâncias, prejuízo cognitivo e comprometimento funcional. O prejuízo causado pela doença parece estar mais relacionado à recorrência dos episódios do que à gravidade de um dado episódio (54).

Dois aspectos clínicos do TB são especialmente importantes: a maior ocorrência de doenças clínicas e os déficits cognitivos a longo prazo. Quanto ao primeiro aspecto, sabe-se que os pacientes bipolares apresentam determinadas doenças clínicas, como doenças cardiovasculares, câncer e problemas de tireóide em uma proporção maior que

o resto da população (55,56). Parte desse excesso de mortalidade pode ser devido aos efeitos colaterais de medicamentos usados no tratamento e a diferenças no estilo de vida, como, por exemplo, menor procura por cuidados preventivos de saúde (57). Porém, mesmo quando esses fatores são controlados, o TB permanece como um fator de risco independente para mortalidade cardiovascular (58). Um grande estudo suíço envolvendo mais de 15.000 pacientes bipolares seguidos por 30 anos revelou que a mortalidade por doenças cardiovasculares era a causa de morte mais comum entre pacientes bipolares, seguida do suicídio e do câncer (59). O TB também tem sido associado à maior ocorrência de síndome metabólica (60). Esta é definida como uma síndrome complexa composta por um conjunto de fatores de risco cardiovasculares que são usualmente associados a um depósito central de gordura e a resistência insulínica (61). Seus principais componentes são: obesidade abdominal, alteração no metabolismo da glicose, como hiperglicemia ou diabete mellitus tipo 2, elevação da pressão arterial e dislipidemia (62). Estudos em várias regiões do mundo reportam taxas de síndrome metabólica em cerca de 30% dos portadores de TB (63,64,65,66,67).

No que diz respeito ao funcionamento neuropsicológico, pacientes bipolares parecerm apresentar déficits de memória e de função executiva, mesmo em eutimia (68,69). Uma das funções cognitivas mais estudadas tanto em bipolares eutímicos como em sintomáticos são os processos mnésticos, principalmente no que se refere à habilidade de aprendizagem para estímulos áudio-verbais. Tarefas que requerem aprendizagem de um material novo são realizadas com sucesso se, além de possuir a condição de memorizar, o indivíduo tentar aplicar alguma estratégia para organizar a informação. Os resultados dos estudos realizados até o momento deixam claro que a capacidade de reter informações está preservada no TB, porém há uma dificuldade para

melhorar o desempenho frente à repetição de estímulos devido à inabilidade dos pacientes em aplicar uma estratégia para organizar a informação (70,71,72,73, 74,75,76, 77,78,79,80,81). O baixo desempenho em provas de memória verbal é sugestivo de envolvimento de estruturas frontais (dificuldade para aplicar estratégias) e hipocampais (dificuldade para memorizar informações novas) (82). Em relação à flexibilidade mental e à capacidade para formar conceitos, diversos estudos relataram que bipolares tinham dificuldade para formar categorias em um teste de combinar cartas de acordo com um critério não revelado no momento da instrução *Wisconsin Card Sorting Test* (WCST) (71,82). Uma meta-análise da literatura concernente à disfunção cognitiva corrobora a ideia de que dificuldades no funcionamento executivo e dificuldades na memória verbal são os achados mais fortemente associados ao TB (73). Bipolares na fase eutímica apresentam prejuízo na memória verbal e visuoespacial (mais especificamente quanto à aplicação de estratégias), bem como em outros componentes do funcionamento executivo, embora exista certa inconsistência em alguns dos achados decorrentes de variáveis como tempo da doença, medicações adotadas, número de internações ou de episódios agudos que o sujeito sofre ao longo da doença (81).

2.2 Aspectos neurobiológicos do TB

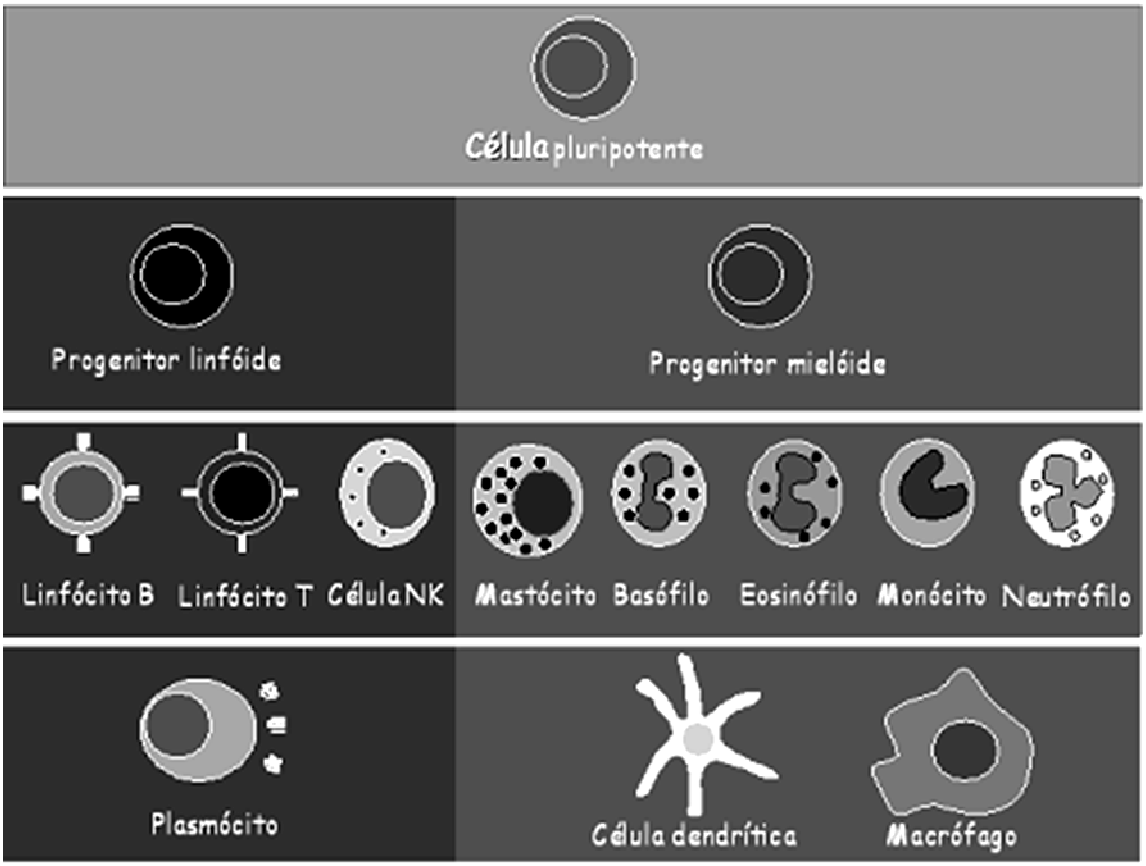
As bases biológicas do TB incluem aspectos relacionados à genética (83,84), a vias neuro-hormonais (85,86,87,88), à neurotransmissão, a vias de transdução de sinal (89,90), à regulação da expressão gênica (91,92,93) e a aspectos relacionados à regulação da neuroplasticidade (94,95).

De fato, estudos recentes descrevem que prejuízos na neuroplasticidade e na regulação da sobrevida neuronal são um evento central na patofisiologia do TB e, possivelmente, explicam os efeitos tardios da doença (96,97,98,99,100). A neuroplasticidade refere-se à capacidade do SNC de modificar-se e de sustentar essas mudanças, tendo como objetivo a melhor adaptação ao ambiente externo e interno (97). As diferentes modificações celulares e moleculares que compõem a neuroplasticidade te, como objetivo último, preservar o neurônios e glia e favorecer sua sobrevida e seu bom funcionamento (97). A sobrevida neuronal é influenciada por diversos fatores, incluindo a ação orquestrada de neurotransmissores, hormônios, neurotrofinas e também citocinas.

3 CITOCINAS INFLAMATÓRIAS

As citocinas são proteínas ou glicoproteínas envolvidas na ação do sistema imunológico. O sistema imunológico é um sistema de defesa do organismo contra agressões variadas, especialmente patógenos e células tumorais (101). A imunidade depende de três linhas de defesa: as barreiras físicas (como a pele e as secreções, por exemplo), a imunidade inata e a imunidade adaptativa (101). A imunidade inata é o sistema mais antigo do ponto de vista evolutivo, é a primeira linha de defesa do hospedeiro e é relativamente inespecífica (102). Já a imunidade adaptativa é específica para o agente agressor e depende da produção de anticorpos contra ele (102). Ambas as linhas dependem da ação coordenada de vários subtipos de leucócitos, como os linfócitos, os monócitos e os granulócitos (neutrófilos, eosinófilos e basófilos). As células envolvidas na resposta imunológica encontram-se sumarizadas na Figura 1.

Figura 1 - Resumo das células envolvidas na resposta imunológica



O sistema imunológico, para exercer sua função de defesa, necessariamente deve estar disperso pelo organismo. Desta forma, quando ocorre, por exemplo, a penetração de um agente estranho, quase que imediatamente o sistema imunológico é ativado, os leucócitos migram para o local da agressão e lá exercem prioritariamente a função de fagocitose desses agentes. A atividade de fagocitose é realizada, principalmente, pelos macrófagos e também pelos granulócitos. A partir da fagocitose, os macrófagos digerem esses elementos e transportam, para a sua superfície, partículas desses elementos (antígenos). Após esse processo, os linfócitos podem reconhecer esses elementos e dar continuidade à resposta imune (103).

Existem dois tipos de linfócitos: os linfócitos B e os linfócitos T. O linfócito B produz e secreta moléculas denominadas anticorpos. As células T reconhecem o antígeno na superfíce do macrófago e passam a secretar mediadores da resposta inflamatória, denominados citocinas. Os linfócitos T que exercem essa função são chamados de T auxiliares (T *helper*). As citocinas estimulam a multiplicação, a diferenciação e a secreção de anticorpos pelo linfócito B, que, quando maduro, passa a ser donominado plasmócito (103,104).

Os anticorpos produzidos são liberados na circulação, onde se ligam aos antígenos. A simples ligação do anticorpo aos antígenos nem sempre é suficiente para eliminar os antígenos. Em geral, múltiplos anticorpos ligam-se aos antígenos, ativam proteínas do sistema complemento, formando um complexo que lisa a células estranhas ou facilita a fagocitose desses antígenos pelos macrófagos (103,104).

A inflamação é a resposta dos tecidos a agressões locais, depende da imunidade inata e envolve leucócitos e mediadores inflamatórios, como as citocinas (101). Ela tem como objetivo a mobilização das defesas corporais, a localização das lesões, a destruição de patógenos e a facilitação do reparo tecidual (101). A resposta inflamatória é iniciada pela liberação de mediadores químicos pelo tecido destruído, antígenos e proteínas do sistema complemento. Macrófagos e neutrófilos migram rapidamente para o sítio da agressão e lá iniciam o processo de fagocitose com a produção de diversos mediadores com o objetivo de atrair outros leucócitos e amplificar a reposta (102).

O desencadeamento da resposta inflamatória leva à produção local e sistêmica de diferentes citocinas que podem ter efeitos locais e sistêmicos que explicam alguns sinais das doenças agudas (102). Os efeitos sistêmicos incluem alterações na temperatura corporal, fluxo sanguíneo e tráfego celular e níveis séricos de proteínas.

Esses eventos coordenados, coletivamente denominados “reação de fase aguda” são atribuídos à produção maciça de citocinas (102). Classicamente, consideram-se como mediadores da inflamação as citocinas TNF-alfa, a IL-1 e a interleucina-6 (IL-6), embora recentemente diversas outras citocinas tenham sido implicadas (102).

Resumidamente, as citocinas podem ser definidas como orquestradores da imunidade inata e adaptativa, regulado diversas funções celulares (entre elas, a proliferação, sobrevida e maturação) (105). Um resumo das citocinas mais relevantes está demonstrado na Tabela 1.

Tabela 1 - Fontes mais importantes e atividades das principais citocinas

Citocina	Fonte primária	Atividade biológica primária
IL-1	Macrófagos	Mediador inflamatório, reagente de fase aguda, amplificação da resposta imune
IL-2	Linfócitos T	Ativação das células T
IL-3	Linfócitos T	Fator de crescimento hematopoiético inespecífico
IL-4	Linfócitos T	Ativação de células T e B, fator de crescimento de mastócitos
IL-5	Linfócitos T	Fator de crescimento de células B, promove produção de IgA, crescimento e diferenciação de eosinófilos
IL-6	Macrófagos e linfócitos T	Diferenciação de células B, reagente de fase aguda
IL-7	Estroma do baço e timo	Fator de crescimento em fases muito precoces dos linfócitos T e B

IL-8	Macrófagos e linfócitos T	Fator quimiotático para neutrófilos e linfócitos
IL-9	Linfócitos T	Fator hematopoiético amplificador
IL-10	Linfócitos T	Inibe a ativação da resposta celular, inibe a produção de citocinas pelos monócitos e macrófagos
TNF-alfa	Macrófagos, linfócitos T, mastócitos, células NK.	Sobrepõe-se à atividade da IL-1, mas com mais atividade antitumoral
INF-alfa, INF-beta, INF-gama	Linfócitos B, macrófagos, fibroblastos, células epiteliais, linfócitos T	Atividade antiviral e antitumoral, ativa macrófagos, aumenta atividade de linfócitos T citotóxicos e células NK
GM-CSF	Linfócitos T, fibroblastos, células endoteliais	É fator de crescimento para granulócitos, macrófagos e eosinófilos, ativa fagocitose de neutrófilos, aumenta citotoxicidade mediada por eosinófilos, promove liberação de histamina pelos basófilos

GM-CSF: Fator estimulador de colônias de granulócitos e macrófagos; NK: *natural killer cells*.

3.1 Quimiocinas

As quimiocinas fazem parte de uma família especializada de citocinas que funcionam como potentes mediadores ou reguladores da inflamação e possuem a habilidade específica de recrutar e ativar subpopulações específicas de leucócitos (106). Sua principal função é a quimiotaxia, ou seja, a atração de outras células para o local onde está ocorrendo a inflamação (106). As quimiocinas são divididas em quatro subfamílias dependendo da posição de seus resíduos de cisteína. Essas subfamílias são CC, CXC, CX3C e XC. Na subfamília CXC um aminoácido separa as primeiras duas cisteínas, enquanto na subfamília CC os primeiros dois resíduos de cisteína são adjacentes um ao outro. Cada subfamília possui seu próprio receptor e tem funções diferenciadas uma da outra. As quimiocinas CXC são quimiotáxicas para neutrófilos, enquanto as CC não agem nesse último grupo celular, mas atraem monócitos, basófilos e linfócitos (107).

4 CITOCINAS E TRANSTORNO BIPOLAR

O conhecimento crescente das interações entre o SNC e o sistema imune e as evidências de aumento da atividade pró-inflamatória na depressão e na esquizofrenia, levaram diferentes pesquisadores a voltar suas atenções para a possibilidade de um efeito semelhante no TB. Nos últimos cinco anos, alguns estudos abordando possíveis diferenças entre os níveis periféricos de citocinas de portadores de TB e de sujeitos sem a doença foram realizados, e seus resultados serão descritos a seguir.

Os estudos realizados até o momento demonstram que pacientes bipolares apresentando episódios de depressão bipolar ou de mania tem um aumento geral na atividade inflamatória, expressa pelo aumento de citocinas inflamatórias no soro. A maioria dos estudos mostra uma hiperatividade de células da linhagem T *helper* 1 (Th1), com aumentos significativos de TNF-alfa no soro de pacientes bipolares durante episódios de mania (108,109) ou depressão (108,109) quando comparados a controles saudáveis. Resultados similares a estes também foram encontrados por Kim et al. (2007), que descreveram aumento da produção de IL-6 e TNF-alfa em pessoas com TB durante a mania (110). Nesses pacientes, os níveis de IL-6 retornavam ao normal após seis semanas de tratamento estabilizadores do humor, mas os níveis de TNF-alfa se mantinham elevados. Com base neste achado, os autores sugeriram que a IL-6 elevada poderia ser um marcador de estados maníacos, enquanto o TNF-alfa poderia representar um marcador mais duradouro da presença do TB (110).

Em outro estudo recente, O'Brien et al. (108) descreveram que tanto a mania quanto a depressão bipolar são associadas a elevação na produção de citocinas pró-inflamatórias, como a IL-6 e a IL-8, mesmo com o uso de medicação estabilizadora do

humor e antipsicótica (108). Os mesmos autores não encontraram diferenças nas concentrações da citocina anti-inflamatória IL-10 e no cortisol (108). Quanto a outras citocinas, Ortiz-Domínguez et al. relataram redução da IL-12 na mania e na depressão, aumento da IL-6 na mania e da IL-4 na depressão (109). Um único estudo comparou os níveis de IFN-gama em pacientes bipolares em eutimia com controles (111). Um resumo dos estudos abordando citocinas no TB está descrito na Tabela 2.

Tabela 2 - Marcadores de resposta inflamatória que estão elevados no transtorno bipolar

Citocinas pró-inflamatórias			
	IL-6	Elevada na mania	Kim et al. (110)
	IL-8	Elevada na mania e depressão	O'Brien et al. (108)
	TNF-alfa	Elevado na mania e depressão	O'Brien et al. (108); Kim et al. (110)
Citoquinas anti-inflamatórias	IL-4	Reduzida na mania	Kim et al. (110)
Receptores	RIL-2s	Elevado na mania	Tsai et al. (112)

5 CITOCINAS NA PATOFISIOLOGIA DO TRANSTORNO BIPOLAR

5.1 Efeitos das citocinas no metabolismo de neurotransmissores

No momento em que as citocinas atingem as células do tecido cerebral, elas têm a capacidade de influenciar a síntese, liberação e recaptação de diversos neurotransmissores envolvidos na fisiopatogenia do TB, incluindo as monoaminas (113). Existe uma extensa literatura com estudos animais demonstrando que as citocinas ou indutores de sua liberação possuem a habilidade de afetar profundamente o metabolismo da serotonina, noradrenalina e dopamina (114, 115,116,117).

Ainda há dúvidas sobre o mecanismo envolvido nesse processo, embora uma via que vem recebendo atenção crescente seja a via da indolamina 2,3 dioxigenase (IDO). A IDO é uma enzima largamente distribuída em diversos tipos celulares e é induzida preferencialmente pelo INF-gama (118), embora evidências recentes indiquem que ela pode ser ativada por diversos sinais de vias inflamatórias, incluindo o ativador de transcrição 1a (STAT1a), o fator regulatório do INF (IRF-1), o NFkappaB e a proteína quinase ativada por mitógeno p38 (119). A IDO é uma enzima que cliva o triptofano (TRP), o aminoácido precursor da serotonina em quinurenina (KYN), reduzindo a disponibilidade de triptofano na produção do neurotransmissor. A velocidade em que essa quebra ocorre é importante para determinar a quantidade de serotonina produzida (120). Uma das evidências que indicam o envolvimento da IDO nos transtornos de humor provém da demonstração de redução de TRP e aumento de KYN no sangue periférico de pacientes em imunoterapia com interferon e da correlação dessas alterações com sintomas depressivos, ansiosos e cognitivos (121). Da mesma forma, a inibição da IDO também inibiu o desenvolvimento de comportamentos depressivos

induzidos pelo lipopolissacarídio (LPS) em ratos (122). É interessante notar que a indução da IDO por citocinas e a consequente produção de KIN pode ter um efeito no humor independente da redução da serotonina. Por exemplo, a administração de KYN sozinha já é suficiente para induzir comportamentos depressivos em ratos (122). Uma das possíveis explicações para isso advém do fato de que a KIN é preferencialmente convertida em ácido quinureico (KA) em astrócitos e ácido quinolínico (QUIN) na micróglia (120). O ácido quinureico possui a habilidade de inibir a liberação de glutamato, que, por sua vez, inibirá a liberação de dopamina, que é parcialmente regulada pela atividade glutamatérgica (123). Em contraste, o QUIN promove a liberação de glutamato, a ativação de receptores NMDA e o aumento do estresse oxidativo, contribuindo para neurotoxicidade (120,124,125). Assim, o balanço entre o KA e a QUIN pode determinar o efeito das citocinas no SNC e continua sendo uma área importante de investigação.

As citocinas também podem influenciar a síntese de dopamina (DA). Por exemplo, em ratos, a injeção de INF-alfa reduziu as concentrações centrais de tetra-hidrobiopterina (BH4) e DA em associação com a estimulação da produção de óxido nítrico (NO) (126). A BH4 é um importante cofator da enzima tirosina hidroxilase, que converte a tirosina em L-3,4-dihidroxifenilalanina (L-DOPA) e é o passo limitante da síntese de DA. A BH4 também é requerida para a síntese de NO, e o aumento da síntese de NO é associado à redução da síntese de DA (126). A ativação da micróglia é associada a um aumento da síntese de NO (127), sugerindo que a influência das citocinas na BH4 via NO pode ser um mecanismo comum pelo qual as citocinas reduzem a atividade dopaminérgica em algumas regiões cerebrais.

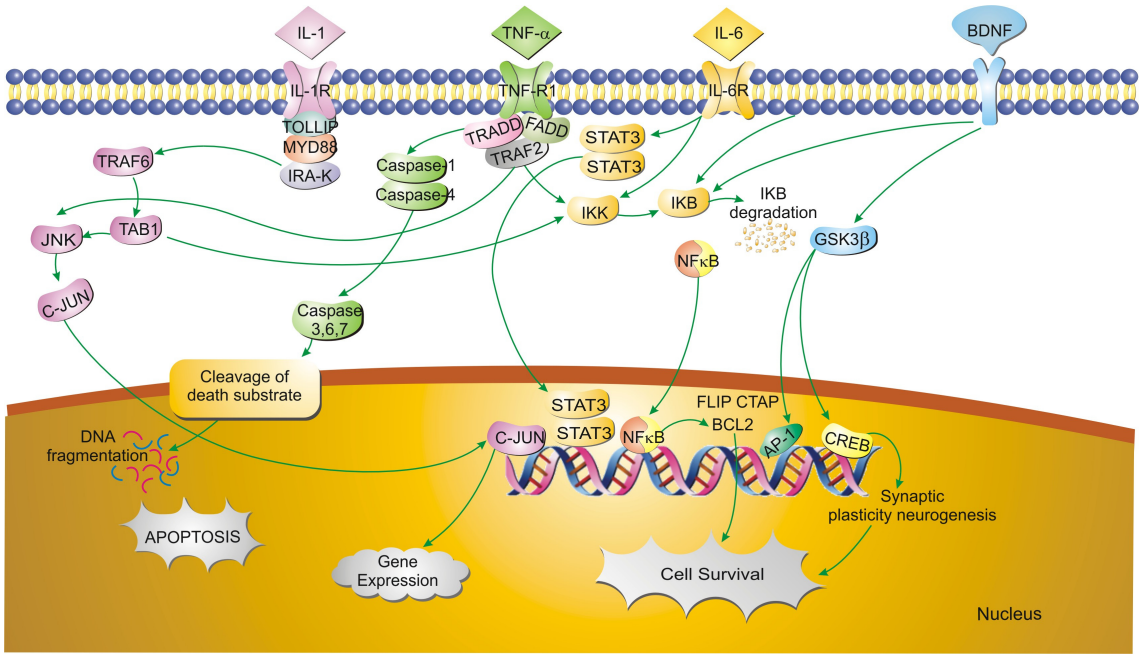
As citocinas e suas vias de transmissão de sinal também podem influenciar a recaptação de monoaminas (113). As vias das MAP-quinases, incluindo a p38 e a ERK 1/2, que mediam os efeitos das citocinas na proliferação, diferenciação e apoptose, e a expressão gênica de mediadores inflamatórios têm se mostrado capazes de influenciar a recaptação sináptica da noradrenalina, serotonina e dopamina (128,129,130). A IL-1 e o TNF-alfa, por exemplo, aumentam significativamente a recaptação de serotonina através da MAP-quinase p38 (130). Corroborando estes achados, dados recentes de estudos em humanos revelam que a administração de INF-alfa reduziu a concentração de ácido 5-hidróxi-indolacético (5-HIAA) e aumentou a de IL-6 no liquor, e que esse fenômeno se correlacionava com humor deprimido (21). Levando em consideração todos esses achados, pode-se concluir que as citocinas exercem um efeito duplo, influenciando tanto a síntese como a recaptação de monoaminas e contribuindo, assim, para reduzir sua biodisponibilidade (28).

5.2 Efeitos das citocinas na neuroplasticidade

No SNC, as citocinas apresentam um papel funcional complexo. Sob condições fisiológicas, citocinas inflamatórias, como a IL-1, IL-6 e o TNF-alfa são importantes fatores tróficos, favorecem a neurogênese e estão associados ao funcionamento cognitivo normal e à consolidação da memória (131,132). A citocina mais estudada é o TNF-alfa. Nos neurônios e na micróglia, o TNF-alfa induz a apoptose quando se liga ao receptor TNF-R1. O TNF-R1 contém uma sequência localizada no citoplasma denominada *death domain*. Essa sequência se liga a uma proteína adaptadora chamada TNF *receptor associated death domain* (TRADD). A TRADD, então, recruta a *Fas-*

associated death domain (FADD), que ativa a cascata das caspases, resultando em degradação do DNA e morte celular. A IL-1 tem uma ação similar (133). Essa sequência de eventos está ilustrada na Figura 2.

Figura 2 - Principais vias de sinalização de citocinas envolvidas na regulação da apoptose.



AP-1: *activator protein 1*; BCL2: *B-cell CLL/lymphoma 2*; BDNF: *brain derived neurotrophic factor*; c-JNK: *c-Jun N-terminal kinases*; CREB: *cAMP response element binding*; FADD: *Fas-associated protein with death domain*; FLIP: *FLICE-like inhibitory protein*; GSK3beta: *glycogen synthase kinase-3 beta*; IKB: *inhibitor of KappaB*; IL-1: interleucina-1; IL-1R: receptor de interleucina-2; IL-6: Interleucina-6; IL-6R: *interleukin-6 receptor*; IRA-K: *interleukin-1 receptor-associated kinase*; JNK: *jun N-terminal kinases*; MYD88: *myeloid differentiation primary response gene* (88); NFκB: *nuclear factor kappa B*; STAT-3: *signal transducer and activator of transcription 3* (duas subunidades); TAB1: *transforming growth factor-activated*

protein kinase 1-binding protein 1; TNF-alfa: *tumor necrosis factor-alpha*; TOLLIP: *toll interacting protein*; TRADD: *TNFR-associated via death domain*; TRAF2: *TNF receptor-associated factor 2*; TRAF6: *TNF receptor associated factor 6*.

A esse respeito, McDonald e colaboradores (2003) demonstraram que, ao incubar células PC12 (semelhantes a neurônios), com TNF-alfa, induziam a atividade da caspase-8 e da caspase-3, um efeito compreensível tendo em vista o efeito neurotóxico dessa citocina (133). Embora as áreas cerebrais exatas em que o efeito tóxico do TNF-alfa é mais pronunciado ainda estejam sendo objeto de estudo, pesquisas em animais mostram maior densidade de receptores no hipocampo e no hipotálamo (134). A expressão do TNF-alfa já foi documentada em astrócitos, micróglia e neurônios em condições fisiológicas e na inflamação (134).

O efeito neurotóxico do TNF-alfa nas células gliais é especialmente importante por causa da marcada redução das células gliais no córtex prefrontal de pacientes bipolares. É sabido que a glia exerce um papel crítico na regulação da concentração de glutamato e na produção e liberação de fatores neurotróficos e, por isso, pode ser um importante fator envolvido no balanço entre apoptose e sobrevida celular (135).

Apesar da bem descrita ação neurotóxica do TNF-alfa, em condições normais, o cérebro possui um mecanismo regulatório para limitar esse efeito, mediado pelo NFkappaB. O NFkappaB é um fator de transcrição presente em todos os tipos celulares, incluindo neurônios e células gliais. Um importante *insight* na função do NFkappaB deriva da observação de que ratos *knockout* para Rel A (uma subunidade do NFkappaB) morrem durante o estado embrionário por apoptose hepática extensa. Além disso, esses ratos demonstram aumento da sensibilidade celular à morte pelo TNF-alfa (136). O

NFkappaB está presente em axônios, dendritos e terminais sinápticos. É composto por duas unidades e, normalmente, é retido no citoplasma devido à sua ligação com a proteína inibitória IkappaB. A fosforilação, ubiquitinação e proteólise da IkappaB permite que o NFkappaB se desloque para o núcleo e induza a transcrição gênica (137). No núcleo, o NFkappaB se liga a regiões promotoras específicas de um grupo de genes que protege a célula da apoptose (138). Diversos sinais ativam a via do NFkappaB em neurônios, como o TNF-alfa, já mencionado, o glutamato, neurotrofinas como o *nerve growth factor* (NGF) e o *activity-dependent neurotrophic factor* (ADNF), precursor amiloide, moléculas de adesão (139), glucocorticoides, produtos de vírus e bactérias e espécies reativas de oxigênio. Dentre os genes que tem sua transcrição estimulada pelo NFkappaB, estão os do grupo dos *cellular inhibitors of apoptosis* (cIAP), BcL2 e BcL-xL, TRAF1/TRAF2 e superóxido dismutase (SOD) (140).

É interessante notar que o NFkappaB tem sido considerado como um mediador chave do efeito de tolerância cerebral. Quando o cérebro é submetido a estímulos nocivos aplicados cerca, mas abaixo do limiar de morte celular, uma resposta adaptativa se desenvolve e protege o tecido cerebral contra estímulos subsequentes da mesma modalidade (tolerância) ou de outra modalidade (tolerância cruzada).

O efeito do NFkappaB, porém, é dependente do tipo celular. A ativação do NFkappaB na micróglia, diferentemente dos neurônios, pode indiretamente promover a morte neuronal. A micróglia e os astrócitos respondem ao aumento do NFkappaB com a produção de grandes quantidades de espécies reativas de oxigênio, excitotoxinas e mais citocinas inflamatórias (139). É interessante notar que as neurotrofinas e as citocinas podem cooperar na sinalização intracelular. Quando células PC12 são coestimuladas com TNF-alfa e NGF ou *brain derived neurotrophic factor* (BDNF), a translocação

nuclear aumenta grandemente, enquanto que nem o NGF nem o BDNF induzem a translocação nuclear de NFkappaB (138). Assim, pode-se presumir que, quando neurônios são expostos a mediadores inflamatórios, a presença de BDNF e NGF pode limitar o dano inflamatório. Na presença de baixos níveis de BDNF, o dano inflamatório não consegue ser contido.

5.3 Citocinas e desempenho em testes neuropsicológicos no TB

A literatura vem demonstrando que, dentre as múltiplas funções das citocinas, está a regulação de funções complexas, como a cognição (141,142,143,144). Em condições fisiológicas, citocinas pró-inflamatórias como IL-1, IL-6 e o TNF-alfa são importantes para prover suporte trófico aos neurônios e estimular a neurogênese. Em experimentos animais, tem se demonstrado que essas citocinas contribuem também para a formação da memória (145,146,147). Em estudos com ratos geneticamente modificados foi demonstrado que a carência de IL-6, por exemplo, está associada a prejuízos na memória dependente e independente do hipocampo (148). Especificamente, a IL-1-beta, IL-6 e o TNF-alfa têm sido associados a declínio cognitivo e a demência em diversos estudos transversais e prospectivos (144,149,150,151). Curiosamente, tanto a expressão excessiva quanto a ausência das citocinas influenciam a consolidação da memória dependente do hipocampo e a plasticidade sináptica nessa região (152).

Embora continue desconhecido o mecanismo exato pelo qual as citocinas estão envolvidas nos processos celulares e moleculares implicados na formação da memória, avanços significativos têm sido obtidos recentemente nesse campo. Sob condições

ambientais adversas, a liberação de citocinas pró-inflamatórias tem um papel benéfico. A IL-1, o TNF-alfa e a IL-6 podem, potencialmente, facilitar a consolidação da memória de situações prejudiciais ao indivíduo e reforçar os esforços do indivíduo em evitar novas exposições. De fato, dados de pesquisa demonstram que a IL-1 impacta a consolidação da memória dependente do hipocampo por meio da ativação dos seus receptores e induz a neuroplasticidade hipocampal (153). Entretanto, se houver estímulos estressores demasiadamente intensos ou sustentados, ou se a resposta inflamatória estiver desregulada, uma quantidade excessiva de citocinas será liberada e poderá produzir dano em regiões cerebrais específicas, com implicações comportamentais, neuroendócrinas ou clínicas de longo prazo. Assim, a liberação de citocinas inflamatórias pode potencialmente explicar a influência do estresse e dos eventos vitais adversos como fatores etiológicos importantes no início do curso da doença (154), bem como os efeitos deletérios do estresse cumulativo no curso do TB (155).

5.4 Citocinas como mediadores da associação entre TB e doenças clínicas

Os portadores de transtornos do humor, quando comparados com a população em geral, apresentam maior mortalidade, principalmente por doenças cardiovasculares (156,157). O TB, de forma semelhante à depressão, é associado a uma redução potencial de 25 a 30 anos de vida (158). A taxa de síndrome metabólica é significativamente elevada em pacientes bipolares, com alta prevalência de obesidade, hipertrigliceridemia e baixos níveis de colesterol HDL (159), bem como de diabetes tipo 2, disfunção tireoidiana e hipertensão (160).

As causas do aumento do risco cardiometabólico nessa população não são completamente entendidas, mas possivelmente incluam fatores não relacionados à doença, como pobreza e dificuldade de acesso a cuidados médicos, bem como efeitos adversos de medicações psicotrópicas (158). A inflamação é um antecedente de doenças cardiovasculares e pode predizer, independentemente, mortalidade cardiovascular em pessoas que sofreram isquemia cardíaca (161,162,163). A transposição desses dados para o TB sugere que a inflamação pode ser o elo que liga essa doença à sobrecarga de condições médicas gerais, especialmente com o avanço da idade e a duração da doença (159).

Apesar disso, persistem dúvidas acerca de qual é a fonte das citocinas inflamatórias periféricas ou se existe algum influxo de citocinas entre o SNC e o sangue. Embora não exista uma resposta definitiva para essas questões, elas estão no cerne do funcionamento do cérebro e de sua interação com o resto do organismo. O mais importante tipo celular que mantém a barreira hematoencefálica são os astrócitos. Os processos podais dos astrócitos se enrolam nos capilares cerebrais regulando a relação entre o SNC e a periferia (164,165,166,167). Quando o cérebro está submetido a algum processo inflamatório, algumas citocinas, como a IL-1, têm o potencial de induzir a transcrição de fatores regulatórios da função endotelial, levando a modificações na permeabilidade vascular por afrouxamento da junção entre as células endoteliais (168), acabando, finalmente, por alterar a função da barreira hematoencefálica (169). O dano astrocitário é um achado bem documentado no TB (170,171,172,173), e é possível que esse mecanismo provoque ruptura da barreira hematoencefálica e aumento do fluxo de citocinas entre o SNC e a circulação sistêmica.

Assim, em função desses achados, o TB tem sido considerado modernamente não mais como uma doença cerebral, mas como uma doença sistêmica (159,174).

6 EFEITO DOS ESTABILIZADORES DO HUMOR NA RESPOSTA INFLAMATÓRIA

Os dados existentes sobre a atividade imunomodulatória do lítio são múltiplos e, nem sempre, convergentes. Pacientes bipolares em uso crônico de lítio apresentam menor número de polimorfonucleares secretores de IL-2, IL-6, IL-10 e INF-gama em relação a controles não bipolares (111).

Em relação ao ácido valproico, alguns estudos têm demonstrado que esse fármaco pode ter um efeito final na redução da inflamação no SNC. Chen et al. (175) demonstraram que o ácido valproico induz a apoptose de células da micróglia em resposta à neurotoxicidade por dopamina. Este mecanismo seria responsável por um efeito neuroprotetor do ácido valproico.

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8 JUSTIFICATIVA

O TB é uma doença comum e potencialmente fatal, e as medicações atualmente disponíveis para o seu tratamento produzem uma resposta apenas parcial. Portanto, há uma necessidade premente de indentificar novas vias relevantes na fisiopatogenia do TB que possam vir a ser traduzidas em novos alvos para o desenvolvimento de drogas efetivas nessa patologia. Além disso, a identificação de novos biomarcadores da doença pode fornecer informações úteis na identificação e monitoração da resposta clínica. Um dos biomarcadores potenciais do TB pode ser o dos níveis de citocinas inflamatórias.

A imunologia do TB é pouco explorada, e os estudos publicados até o momento incluem amostras pequenas, a maioria em episódio de humor, e não descrevem os níveis de citocinas inflamatórias em pacientes em todos os estados de humor (mania, depressão e eutimia).

9 OBJETIVOS

Objetivo geral: comparar os níveis de mediadores inflamatórios entre pacientes bipolares e controles.

Objetivo do artigo 2: comparar os níveis periféricos de TNF-alfa, IL-2, IL-4, IL-6, IL-10 e INF-gama entre pacientes bipolares maníacos, deprimidos, eutímicos e voluntários saudáveis.

Objetivo do artigo 3: comparar os níveis periféricos das quimiocinas CCL2 (MCP-1), CCL3 (MIP-1alfa), CXCL8 (IL-8), CXCL 9 (MIG-1), CXCL10 (IP-10), CCL11 (eotaxina) e CCL24 (eotaxina-2) entre pacientes bipolares e voluntários saudáveis.

10 ARTIGO 1

TNF-alpha as a molecular target in bipolar disorder

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TNF-ALPHA AS A MOLECULAR TARGET IN BIPOLAR DISORDER

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Abstract

The pathophysiology of bipolar disorder (BD) is poorly understood. An emerging body of evidence points to impairments in neuroplasticity, cell resilience and neuronal survival as the main neuropathological correlates of BD. It has been suggested that inflammatory cytokines, particularly TNF-α may play a critical role in this process. In the present review we examine the evidence suggesting that TNF-α regulates apoptotic cascades which may be related to neuronal and glial loss in BD. Current evidence suggests that an increase in serum levels of TNF-α takes place during manic and depressive episodes. The present article reviews the therapeutic implications of TNF-α signaling pathways involvement in the pathophysiology of BD.

Introduction

Traditionally, the brain has been considered as an area without contact with immune mediators, protected by the bloodbrain barrier (BBB) (Schiepers et al., 2005; Gosselin and Rivest, 2007). Current information shows that brain tissue is able to engender immune processes and be influenced by them (Schiepers et al., 2005; Kronfol and Remick, 2000). The elucidation of the immunology of the central nervous system (CNS) might offer new insights regarding causes of a number of neuropsychiatric disorders, like Alzheimer's disease, schizophrenia, and mood disorders (Schiepers et al., 2005; Kronfol and Remick, 2000). In the field of mood disorders, the immune system seems to play a particularly important role. The present work will focus on the findings pertaining to the field of bipolar disorder (BD).

Epidemiological studies indicate the participation of both biological and environmental factors in the pathogenesis of BD. Recent studies have described impairments in neuroplasticity and neuronal survival among BD patients (Schloesser et al., 2007). Neuronal survival is influenced by several factors including the orchestrated action of neurotransmitters, hormones, and neurotrophins. Brain derived neurotrophic factor (BDNF) has emerged as a key mediator for synaptic plasticity, neuronal connectivity, and dendritic arborization (Post, 2007). Moreover, some studies reported decreased BDNF levels in the serum of both manic and depressive episodes of BD (Cunha et al., 2006).

In addition, it is now recognized that the long-term outcome of BD is much less favorable than previously thought, with incomplete recovery between episodes, cognitive impairment, and functioning decline (Mur et al., 2007; Martinez-Aran et al.,

2007). It should be noted that morphometric studies have demonstrated that patients with BD have enlarged third and lateral ventricles (Soares et al., 2005), reduced gray matter in hippocampus and cerebellum (Moorhead et al., 2007), and reduced volumes in some areas of the prefrontal cortex (Blumberg et al., 2006; Soares et al., 2005). An increase in the size of the amygdala has also been reported (Beyer and Krishnam, 2002) and is currently considered to be one of the most consistent findings among BD patients (Phillips and Vieta, 2007). Strakowski et al. (1993) reported that even first-episode mania is associated with larger ventricles and differences in gray/white matter distribution when compared to controls, but this neuroanatomical change tends to be more pronounced with repeated episodes (Strakowski et al., 2002a). It has been argued that similar changes may occur among patients with chronic unipolar depression (for a detailed review of this topic see Campbell and McQueen, 2006). Imaging studies suggest that, in both depressive and bipolar disorder, there is a relatively low prefrontal modulation of subcortical structures; yet, limbic subcortical regions appear to differ in BD and unipolar depression (Strakowski et al., 2002b).

There is also strong evidence of the association between untreated BD and increased rates of death by cardiovascular and cerebrovascular events, giving BD a systemic dimension (Angst et al., 2002; Kupfer, 2005). One of the links between mood disorders and general medical diseases could be the increase of proinflammatory cytokines, particularly TNF-α, which is a causal factor in the development and progression of arteriosclerosis (Evans et al., 2005).

2. Function of inflammatory cytokines in the brain

Cytokines are proteins or glucoproteins secreted by immune cells in response to noxious stimulus. Acting together, either locally or systemically in the immune response, they function like signals among immune cells. It is now known that cytokines can be secreted not only by immune cells. Cytokines are frequently regulated in cascades, where the induction of early cytokines serves to increase the production of later cytokines (Kronfol and Remick, 2000). Cytokines interact with the neuroendocrine system, e.g., the hypothalamic–pituitary–adrenocortical system, the autonomic system and the neurotransmitter system (dopamine, serotonin and glutamate) (Kim et al., 2007a).

Cytokines have high molecular weight but they may cross the BBB either through leaky areas or by active transport (Kim et al., 2007a; Pan and Kastin, 1999). In the brain, they act in specific pathways involved in mood, energy, and activity control. One example of these pathways is the glucocorticoid pathway, with some cytokines increasing corticotrophin release hormone (CRH) release in hypothalamus (Irwin and Miller, 2007; Pasic et al., 2003) or disturbing the functioning of glucorticoids receptors (Irwin and Miller, 2007). TNF-α has also been found to significantly up-regulate the activity of the serotonin transporter, an effect that can determine reduced function of serotonergic transmission by the reduction of synaptic availability of serotonin (Irwin and Miller, 2007).

3. Cytokines in mood disorders

There are some studies describing a potential role of cytokines in the pathogenesis of depression. Patients with depression that are otherwise healthy seem to have activated inflammatory pathways, with increased proinflammatory cytokines,

acute-phase proteins, and increased expression of chemokines and adhesion molecules (Raison et al., 2006; Yang et al., 2007, Kim et al., 2007b). In addition, specific depression symptoms could be associated with increased cytokines levels, like suicidal behavior (Kim et al., in press).

In the field of BD the findings are still limited, but there is some data affirming that mania also could be associated with a proinflammatory state. Some studies reported increased proinflammatory cytokines and hyperactivity of T helper cell 1 in bipolar disorder, with significantly higher TNF-α levels in bipolar patients during manic (Kim et al., 2007a; O'Brien et al., 2006) and depressive episodes than normal controls (O'Brien et al., 2006). Kim et al. (2007a) reported increased production of interleukin-6 and TNF-α during mania when compared with nonbipolar controls. Among such patients, interleukin-6 levels returned to the baseline after 6 weeks of treatment with mood stabilizers, but TNF-α levels continued high. Accordingly, the authors considered that interleukin-6 is a manic state marker, but TNF-α could be an enduring change (Kim et al., 2007a).

In other recent study, O'Brien et al. (2006) described that both mania and bipolar depression are associated with increased production of proinflammatory cytokines interleukin-6, interleukin-8 and TNF-α, even with the use of mood stabilizers or antipsychotic medication. No difference was observed in the concentration of anti-inflammatory cytokine interleukin-10 or in cortisol concentrations between manic subjects and controls. However interleukin-10 production was found to positively correlate with cortisol concentration in mania. The authors considered a possible feedback axis between cortisol and proinflammatory cytokines.

4. TNF-α and bipolar disorder

TNF-α is a 157 amino acid cytokine and is produced in response to injury and inflammatory or infectious stimuli by macrophages, lymphocytes, neutrophils, and structural cells, including fibroblast, smooth muscle cells (Balakumar and Singh, 2006), astrocytes and microglia (Kronfol and Remick, 2000). It is considered a proinflammatory molecule, augmenting the immune response to help speed-up the elimination of pathogens and the resolution of the inflammatory challenge (Kronfol and Remick, 2000). TNF-α has several effects, including cytotoxicity, antiviral activity, transcription factor activation, and immune response regulation (Bhardwaj and Aggarwal, 2003).

TNF-α exerts its effects by binding to specific receptors named TNF-R1 and TNF-R2 (Gosselin and Rivest, 2007). TNF-R1 is more abundantly expressed, existing in most tissues and cell types and appears to be the main signaling receptor. The majority of deleterious effects produced by TNF-α seem to be mediated via this receptor. TNF-R1 mediates many actions of TNF-α, including cytokine production, activation of transcription factors like NF-κB, and apoptosis (Bhardwaj and Aggarwal, 2003). Interestingly, TNF-R1 activation can trigger a dual signaling cascade that in different cell types may lead to apoptosis, proliferation, differentiation, or survival (Fig. 1).

Apoptosis is the term used to describe a kind of cellular death characterized by cytoplasmic membrane blebbing, chromatin condensation, DNA rupture, and lastly by cell fragmentation. The sign for apoptosis may be triggered by intracellular or extracellular proteins, but the final effector is always the same: activation of a group of enzymes called caspases that work like a cascade by targeting and cleaving key cellular

structures and promoting cell decomposition. TNF-α is a cell-surface receptor that acts like an extracellular trigger for apoptosis (Bhardwaj and Aggarwal, 2003).

In neurons and microglia, TNF-α induces apoptosis when it binds to TNF-R1. TNF-R1 contains a sequence located in the cytoplasm called death domain. This death domain sequence then binds to the death domain of a cytoplasmic adapter protein called TNF receptor associated death domain (TRADD). TRADD then recruits Fas-associated death domain (FADD). FADD activates caspase-8 and then the rest of the caspase cascade resulting in the degradation ofDNA and cell death. The apical element in caspase cascade is caspase-8 activated by FADD and the last is caspase-3, which is directly responsible for cell degradation (McDonald et al., 2003). McDonald and collaborators (2003) have found increased activity of caspase-8 and caspase-3 in TNF-α incubated PC 12 cells, an understandable result taking into account the neurotoxic effect of this cytokine.

Although the exact cerebral target areas of TNF-α are still being studied, animal research showed high receptor density in the hippocampus and hypothalamus (Pasic et al., 2003). TNF-α expression was documented in astrocytes, microglia, and neurons within the CNS under physiological conditions. Under inflammatory conditions the expression increases substantially (Pasic et al., 2003).

The effect of TNF-α in glial cells is especially important because the finding of marked reduction in glial cells in the prefrontal cortex of bipolar patients is associated with change in structural imaging studies. It is known that glial cells play a critical role in the regulation of the amount of synaptic glutamate concentration, in the production and release of neurotrophic factors, and are strong collaborators in the delicate balance between cellular life and death in the intimacy of the brain (Manji et al., 2003). Some

studies clearly describe the reduction of glial numbers in the prefrontal cortex in both major depression and BD, (Rajkowska, 2000; Rajkowska et al., 2001). In addition, signs of necrosis and apoptosis have been observed in oligodendrocytes in the frontal cortex and caudate nucleus of bipolar patients (Uranova et al., 2004). Others have found that glial cells appear to be smaller in bipolar patients (Brauch et al., 2006) or with larger nuclei, possibly indicating that they are under strain, working harder to compensate the reduction in number of this cell type (Rajkowska, 2002). Once considered as only supportive elements, today glia seems to carry out important roles in the regulation of high order brain functions like synaptic glutamate levels, CNS energy homeostasis, liberation of trophic factors (Manji and Duman, 2001), serotonergic and noradrenergic transmission (Rajkowska, 2000). Moreover, the marked loss of glia cells observed in BD is probably related to the pathophysiology of this disorder. Interestingly, cell loss which has been described in the brain cortex of bipolar patients is significantly different from the cell loss found in classic neurodegenerative disorders, like Huntington's disease. In these cases, there is a marked loss of neurons accompanied by prominent gliosis with dramatic increases in glial cell density. Consequently, the cortex of patients with mood disorders does not exhibit neurodegeneration, but has a disturbance in neuroplasticity and cell resilience (Rajkowska, 2003). Therefore, it is possible that this cell pattern does not reflect a glial reaction to neuronal loss, which must be accompanied by gliosis but, represent a pathology that begins in glia (Rajkowska, 2002).

5. NF-κB modulation of cell survival, apoptosis and TNF-α production

Under normal conditions, the brain must have a regulatory pathway of immune-induced neuronal apoptosis to limit the potential harmful effects of sustained inflammatory reactions. Although TNF-α kills cells (including neurons) by apoptosis, it may also negatively regulate its own ability to induce cell death. This appears to be mediated by the induction of NF-κB. NF-κB is a transcription factor present in all cell types including neuron and glial cells and could have an important function in promoting cell survival. A major insight into the function of NF-κB has been the observation that Rel A (a subunit of NF-κB) knockout mice die in the embryo stage of development as a result of extensive liver apoptosis. Furthermore, cells derived from these mice demonstrate enhanced sensitivity of TNF-α-induced cell death (Sun and Anderson, 2002).

NF-κB is present in axons, dendrites, and synaptic terminals. NF-κB is composed of two subunits and normally retained in the cytoplasm through its association with the inhibitory I-κB. Phosphorilation, ubiquitination, and proteolysis of I-κB allows NF-κB to translocate to the nucleus and induce gene transcription (Sun et al., 2001). In the nucleus, NF-κB binds to specific sequences in the promoter/enhancer regions of a set of genes that prevents cells to undergo apoptosis (Furuno and Nakanishi, 2006). Several signals could activate NF-κB in neurons, like TNF-α (mentioned above), the excitatory neurotransmitter glutamate, nerve growth factor (NGF), activity-dependent neurotrophic factor (ADNF), a secreted form of amyloid precursor protein, cell adhesion molecules (Mattson, 2005), glucocorticosteroids, products of virus and bacteria, and reactive oxygen species.

When activated, NF-κB translocates to the nucleus where it binds to specific sequences of DNA and induces the transcription of genes which are important for cell

survival. Among these genes are cellular inhibitors of apoptosis (cIAP), BcL2 e BcL-xL, TRAF1/TRAF2, superoxide dismutase (SOD) (Mattson and Meffert, 2006). Early indications that NF-κB could promote survival in neurons came from studies of embryonic rat hippocampal cultures. When the neurons were previously incubated with TNF-α they were more resistant to death when exposed to metabolic and excitotoxic insults (Mattson and Meffert, 2006).

Moreover, Blondeau et al. (2001) developed the concept of NF-κB as the key event in brain tolerance. When the brain is submitted to noxious stimuli applied close but below the threshold for cell death there are adaptative responses that protect it against additional stress from the same (tolerance) or other stimuli (cross-tolerance). The authors studied three models of brain injury (seizure, ischemia, and poly-unsaturated fatty acid damage) submitting hippocampal neurons of rats to them. After that, the neurons received a dose of the proconvulsive substance kainic acid or a sublethal 3-min ischemia. The pretreatment caused a rapid increase of NF-κB DNA-binding activity and nuclear translocation of subunits of NF-κB and led to neuronal resistance to subsequent injury. In contrast, pretreatment with a NF-κB inhibitor diethyldithiocarbamate or κB decoy DNA eliminated the nuclear translocation of NF-κB and the neuroprotective effects of sublethal noxious stimuli (Blondeau et al., 2001). Therefore, there is strong evidence that NF-κB is the cell mediator of brain tolerance and the study of this process could open new avenues in the search for therapeutic strategies targeting neuronal protection.

However, the activation of NF-κB by glial cells might indirectly promote neuronal death. Microglia and astrocytes respond to the increase of intracellular NF-κB with the production of large amounts of reactive oxygen species, excitotoxins, and more

proinflammatory cytokines (Mattson, 2005). A potential explicative model concerning the role of NF-κB in neuronal survival is that the activation of NF-κB in neurons could promote their survival and the activation of this transcription factor in glial cells may induce neuronal loss (Mattson, 2005; Mattson and Meffert, 2006).

Interestingly, neurotrophic factors and cytokines could cooperate in intracellular signaling. When PC12 cells are costimulated with TNF-α and NGF or BDNF, the nuclear translocation of NF-κB increases greatly whereas, neither NGF nor BDNF themselves induces NF-κB translocation (Furuno and Nakanishi, 2006). Thus, it can be hypothesized that when neurons are exposed to inflammatory states, the presence of BDNF and NGF might limit the immune injury in the brain. In the presence of low levels of neurotrophic factors, the amplification of inflammatory damage could not be controlled.

6. TNF-α and NF-κB genetics

Considering the potential role of TNF-α and NF-κB in the pathophysiology of BD, it could be beneficial to know if a genetic variation of these proteins may confer risk for BD. There are two studies conducted with Korean participants that analyzed polymorphisms of TNF-α and investigated its relationship with BD. One of these described an association between TNFA*2 allele and BD (Kim et al., 2003) and the other found an association between the −G308A and this condition (Pae et al., 2004). Furthermore, there is a post mortem research investigating the brains of individuals with BD to determine the levels of RNA encoding components of the NF-κB transcription complex. This study demonstrated increased levels of mRNA in the frontal cortex from individuals with BD compared with controls (Sun et al., 2001).

7. TNF-α and cognitive impairment

Cognitive decline has been well documented in bipolar patients and possibly has a negative impact on social functioning and disability. The etiology of cognitive impairment in bipolar disorder is not completely understood but it seems to be related to the number of episodes, the number of hospital admissions, and the duration of the illness. Both depressive and mania episodes related negatively to cognitive performance but mania seems to be more consistently related to a delay in verbal memory and executive functions, these deficits persist even in euthymia (Robinson and Ferrier, 2006).

There are several reports of impairments in cognitive functioning during inflammatory reactions. In animals, increases of proinflammatory cytokines impair spatial learning and disrupt hippocampus dependent memory formation (Larson and Dunn, 2001). Also, several early observations indicate that TNF-α levels increased in cerebral spinal fluid (CSF) of HIV patients and this effect is more pronounced in those with dementia rather than those without. In fact, there is some evidence that TNF-α levels are directly associated with cognitive decline in this population (Seilhean et al., 1997) and that some HIV proteins like Tat are able to reduce neuronal NF-κB activity, which could be a possible pathway for dementia (Sui et al., 2006). TNF-α modulation could be important in others types of dementia, like Alzheimer's disease where TNF-α level in CSF elevated 25- fold when compared to controls and TNF-α levels are correlated with clinical cognitive performance. In Alzheimer's disease, beta-amyloid stimulates TNF-α production by microglia and it could be the mechanism of neurotoxicity. In fact, a preliminary open label study with etanercept, an inhibitor of

TNF-α, reported a significant improvement of the Mini Mental State Examination (MMSE) and other cognitive tests after treatment (Tobinick et al., 2006).

Despite the possibility of TNF-α functioning as a mediator of cognitive impairment in BD, until now, there are no studies evaluating this relationship.

8. Effects of mood stabilizers on TNF-α production and NF-κB transactivation

Regarding the effects of mood stabilizers, one study showed that valproate suppressed TNF-α production and the inhibition of NF-κB production in vitro in human glioma cells (Ichiyama et al., 2000). Thus, it can be hypothesized that valproate could interrupt the amplification of the production of TNF-α in response to the activation of TNF-α/NF-κB in the brain and prevent the release of inflammatory cascades.

It is noteworthy that lithium has two different effects in NF-κB, consistent with the notion that the effects of lithium are cell specific. Treatment of mouse embryonic fibroblasts with lithium decreases TNF-α-induced NF-κB transactivation. In contrast, in neuron-like PC12 cells, lithium increases NF-κB activity. This increase in NF-κB activity in PC12 is associated with decreased apoptosis in these cells, suggesting that lithiuminduced activation of the antiapoptotic molecule of NF-κB contributes to the neuroprotective effects of lithium (Nemeth et al., 2002; Bournat et al., 2000). Also, lithium is a direct inhibitor of glycogen synthase kinase-3β (GSK3β). The activity of GSK3β has a major influence on cell survival, with hyperactive GSK3β increasing the susceptibility of cells to the lethal consequences of a wide variety of insults. It is possible that inhibition of GSK3β accounts for much of lithium's neuroprotective capacity. Furthermore, GSK3β regulates several transcription factors including NF-κB. Bournat et al. (2000) demonstrated an inhibitory influence of GSK3β on the

transcriptional activity of NF-κB in PC 12 cells. When the activity of GSK3β was inhibited, the result was increased cellular survival and reduced apoptosis in a NF-κB-dependent pathway (Jope and Bijur, 2002; Bournat et al., 2000). This effect probably is not lithium-specific and there is some evidence that lamotrigine and valproate have a similar function (Li et al., 2002).

Taking into account the studies mentioned above, it is suggested that traditional mood stabilizer medication might have some immunomodulatory actions among its therapeutic action that were not suspected before.

9. Conclusions

There is strong evidence that bipolar disorder involves abnormalities in multiple aspects of immunity, including TNF-α. Probably this cytokine is not only a secondary marker of the disorder, but it might contribute to the pathophysiology of BD. The exploration of inflammatory mechanisms of BD could lead to a new wave of therapeutic options including anti-inflammatory cytokines inhibition and modification. Although its clinical benefits are still under investigation, the intriguing possibility of developing pharmacological treatments for BD which would be based on the inhibition and or the modulation of the activity of inflammatory cytokines, their receptors, and their specific signal transduction pathways is a promising approach. Such developments could bring the field closer to other areas of medicine where the main goal is the reversion of the underlying pathophysiology, instead of only treating the symptoms. Despite these exciting possibilities, it is important to remember that there are still many metabolic pathways and cytokines effects in brain that remain completely unknown.

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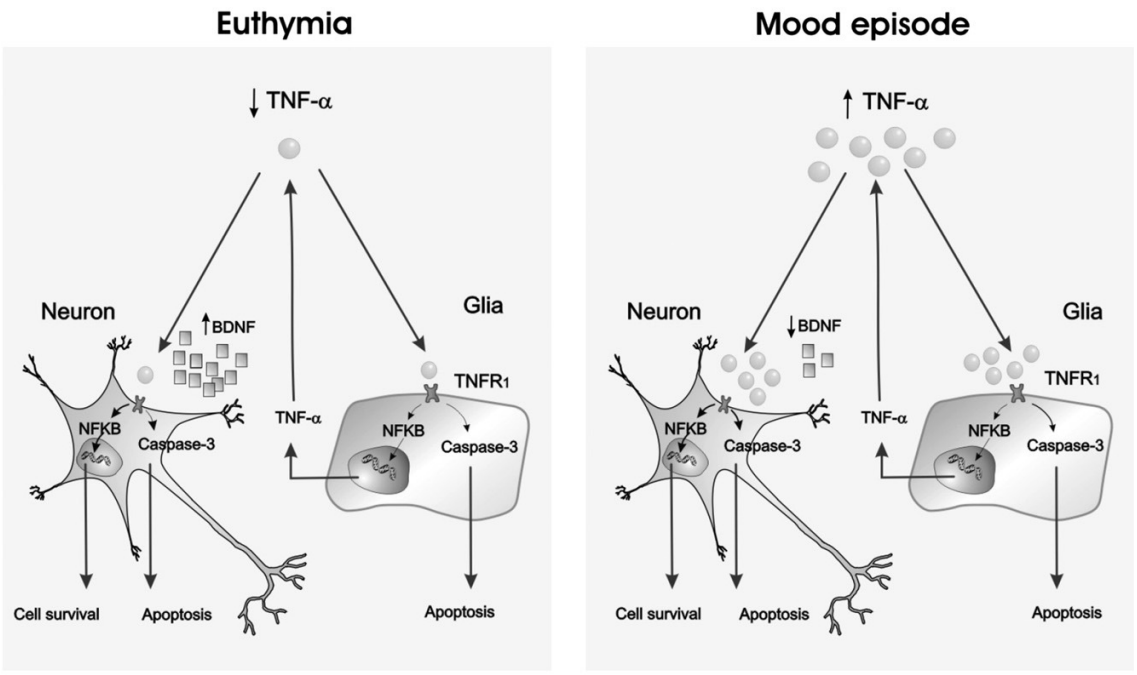
Figure 1. During mood episodes, increased TNF-α levels enhance TNFR1 stimulation in glial cells and this activates both apoptotic routes and TNF-α production. In neurons, cell death mechanisms related to caspase activity are set into motion. In this context, neuronal translocation of NF-κB is shut down due to lower levels BDNF (which have been described during mood episodes by Cunha et al., 2006). Therefore, we postulate that during acute mood episodes the balance between cellular life and death is shifted towards apoptosis.



11 ARTIGO 2

Comparison of cytokine levels in depressed, manic and euthymic patients with bipolar disorder.

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COMPARISON OF CYTOKINE LEVELS IN DEPRESSED, MANIC AND EUTHYMIC PATIENTS WITH BIPOLAR DISORDER

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Abstract

Background: The neurobiology of bipolar disorder is not completely understood. Cytokines have received increasing attention as potential mediators of the interaction with immune, neuroendocrine system and specific pathways involved in mood, energy, and activity control. Previous reports have suggested the association of mania and bipolar depression with a proinflammatory state. However, they did not compare cytokine levels in all phases of bipolar disorder.

Methods: Sixty-one bipolar patients were recruited for assessment of serum cytokine levels. Of these, 14 were in euthymic state, 23 and 24 were in manic and depressive episodes, respectively. A healthy comparison group included 25 healthy volunteers. Cytokines involved in Th1/Th2 balance, such as TNF-α, IL-2, IL-4, IL-6, IL-10, IFN-γ, were examined by flow cytometry.

Results: During mania, proinflammatory cytokines, IL-2, IL-4 and IL-6, were increased in comparison with healthy subjects. Patients in depressive episode showed only increased IL-6 levels. There were no significant differences in cytokine levels between patients in remission and healthy subjects, except for IL-4.Mood symptoms showed a positive correlationwith IL-6 and IL-2.

Discussion: These findings suggest that mania, and to a less extent, depression are associated with a proinflammatory state. These changes seem to be related to mood state, as changes in cytokine profile were more pronounced during acute episodes than in euthymia. This study provides further support to investigate the immune system as a target for future treatment development.

1. Introduction

Bipolar disorder (BD) is a highly prevalent and disabling condition; however the pathophysiology remains largely unknown. In the last decade, the importance of cytokines in neuronal survival was recognized (Brietzke and Kapczinski, 2008), along with the orchestrated action of neurotransmitters, hormones, and neurotrophins (Post, 2007). Recent studies have described impairments in neuroplasticity and neuronal survival in BD (Schloesser et al., 2007). In addition, cytokines can interact with the neuroendocrine system and in specific pathways involved in mood, energy, and activity control (Irwin and Miller, 2007; Pasic et al., 2003). Regardless of their etiology, chronic inflammatory responses in individuals with mood disorders may result in downstream biological damage and contribute to the elevated cardiac and other medical morbidities associated with BD (McEwen, 2003; Simon et al., 2008; Kapczinski et al., 2008).

Despite a growing body of evidence of the involvement of cytokines in psychiatric and neurodegenerative disorders, findings are still limited in BD. There are reports suggesting an association of mania and depression with a proinflammatory state. However, these findings present some inconsistencies, which could be related to sample heterogeneity regarding moodsymptoms, length of illness and effect of medications. As far as we know, previous studies have not compared cytokine levels in all phases of BD. Some studies reported increased proinflammatory cytokines and hyperactivity of T helper cell 1 in BD, with significantly higher TNF-α levels in bipolar patients during manic (Kim et al., 2007; Ortíz-Domingues et al., 2007; O'Brien et al., 2006) or depressive episodes when compared with normal controls (Ortíz-Domingues et al., 2007; O'Brien et al., 2006). Kim et al. (2007) reported increased production of

interleukin-6 (IL-6) and TNF-α during mania when compared with nonbipolar subjects. Among such patients, IL-6 levels returned to the baseline after 6 weeks of treatment with mood stabilizers, but TNF-α level continued high. Accordingly, the authors suggest that IL-6 could be a manic state marker, while TNF-α could be an enduring change (Kim et al., 2007). In another recent study, O'Brien et al. (2006) described that both mania and bipolar depression are associated with increased production of proinflammatory cytokines IL-6, IL-8 and TNF-α, even with the use of mood stabilizers or antipsychotic medication. No difference was observed in the concentration of anti-inflammatory cytokine IL-10 or in cortisol concentrations between manic subjects and controls. In addition, Ortíz-Domingues et al. reported increased TNF-α and reduced IL-2 levels both in mania and depression and increased IL-6 in mania and IL-4 in depression. A single study that has examined IFN-γ levels during euthymia in BD showed decreased levels in comparison with controls, but did not include those with mood episodes (Boufidou et al., 2004).

There are several methods to assess cytokine levels. Usually they are measured using ELISA kits, but other methods such as flow cytometry have been proposed. Actually, previous studies showed that flow cytometry can adequately replace ELISA and PCR in basic research as well as in immune diagnostics (Hemdan, 2008).

Therefore, this study was designed to examine which changes in cytokine levels are associated with mood episodes and which are more enduring changes, being present during euthymic periods. The levels of cytokines involved in Th1/Th2 balance, such as TNF-α, IL-2, IL-4, IL-6, IL-10, IFN-γ, were compared between depressive, manic and euthymic phases of BD. The understanding of inflammatory mechanisms of BD could potentially open avenues for new treatment options and for the investigation of the use

of anti-inflammatory medications in BD (Brietzke and Kapczinski, 2008; Nery et al., 2008).

2. Methods

The present study was approved by the local ethics committee and all subjects provided written informed consent before entering in the study. Sixty-one patients with BD type I were recruited from the Bipolar Disorders Program of Hospital de Clínicas de Porto Alegre, Brazil, and the Inpatient Psychiatric Unit, Hospital Espírita de Porto Alegre, Brazil. Of these, 14 were in euthymic state, 23 and 24 were in manic and depressive episodes, respectively. A comparison group with 25 healthy volunteers was also recruited. Psychiatric diagnosis was based on clinical interview and confirmed with the Structured Clinical Interview for DSM-IV-Axis I (SCID-I). Manic and depressive symptoms were assessed using the Young Mania Rating Scale (YMRS) and the Hamilton Depression Rating Scale (HDRS), respectively. Acute manic or depressive episodes were defined by DSM-IV-TR criteria. Patients were divided into three different groups according to the following criteria: 1) euthymia group should not fulfill DSM-IV criteria for mood episode and should have HDRS and YMRS scores less than 8; 2) mania group should have YMRSN7 and HDRSb7; 3) depression group should have HDRSN7 and YMRSb7. Patients in mixed episodes were not included. The healthy comparison group was screened for psychiatric disorders using SCID-I, non-patient version. Healthy subjects were not on medication, and had no history of major psychiatric disorders, dementia, mental retardation, cancer or tumor in their first-degree relatives. Exclusion criteria to both patients and controls included presence of general

medical conditions associated with changes in inflammatory response, such as HIV/AIDS, rheumatologic diseases, current bacterial or viral disease and current use of more than 10 cigarettes a day. Similarly, none of the groups included individuals in current use of corticosteroids, nonsteroid anti-inflammatory medications, acetylsalicylic acid or immunosuppressive therapy.

Five milliliters of blood were withdrawn from each subject by venipuncture into a free-anticoagulant vacuum tube. The blood was immediately centrifuged at 3000 ×g for 5 min, and serum was kept frozen at −80 °C until assayed. The concentration of serum cytokines was determined by flow cytometry using the BD™ Cytometric Bead Array (CBA) Human Th1/Th2 Cytokine Kit II (BD Biosciences, San Diego, CA). The CBA kit employed here allows for the discrimination of the following cytokines: IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF-α. Sample processing and data analysis were performed according to the manufacturer's instructions. Briefly, serum samples were incubated with the six cytokine capture beads and PE-conjugated detection antibodies for 3 h, at room temperature and protected from light. Afterward, samples were washed and sample data were acquired using a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA). Sample results were generated in graphical and tabular format using the BD CBA Analysis Software (BD Biosciences, San Diego, CA).

3. Statistics

Descriptive analyses are presented in means and percentage. Demographic and clinical characteristics were analyzed using chi-square and ANOVA test as indicated. Cytokine levels showed a nonparametric distribution andwere analyzed with appropriate

tests as indicated in tables; Kruskal–Wallis test and Mann–Whitney test with Bonferroni correction for multiple comparisons (control vs. euthymic, manic and depressed groups).

4. Results

Demographic and clinical characteristics of the sample (n=86) are presented in Table 1. Both groups were homogeneous regarding age, gender, ethnic group and years of schooling. Median and percentiles 25 and 75 for cytokine levels in patients and healthy subjects are shown in Table 2. Regarding duration of disorder, patients in depressive episode and euthymia had a significant longer time of illness (26 years and 21 years, respectively) than manic patients (7 years).

Results demonstrated that only IL-4 levels were increased in euthymic patients compared with healthy subjects (U=141.50, p=0.007, Z=−2.68, ES=−0.43). Manic patients, when compared to controls, had increased levels of IL-2 (U=158.00, p=0.001, Z=−3.03, ES=−0.44), IL-4 (U=171.00, p=0.011, Z=−2.51, ES=−0.36) and IL-6 (U=137.50, p=0.002, Z=−3.23, ES=−0.47). In patients in depressive episode, IL-6 was increased compared with controls (U=187.50, p=0.017, Z=−2.38, ES=−0.34). Except for those described above, there were no differences in IL-2, IL-4 and IL-6 levels. In addition, there were no differences in IL-10, IFNγ and TNF-alpha levels between patients (all groups considered) and healthy subjects (Table 2).

Mood symptoms showed a positive correlation with IL-6 and IL-2. Manic symptoms, as indicated by YMRS scores, had a positive correlation with IL-6 (rs=0.30, p=0.004) and with IL-2 (rs=0.30, p=0.005). Depressive symptoms, as indicated by

HDRS scores, showed a positive correlation only with IL-6 (rs=0.26, p=0.014). Regarding duration of disorder, there was no observed correlation between any cytokine level and years of illness.

5. Discussion

This study revealed increased levels of proinflammatory cytokines IL-2, IL-4 and IL-6 in manic states when compared with healthy control subjects (Table 2), suggesting that mania may be a proinflammatory state. In depressed bipolar patients, IL-6 is increased when compared with control subjects. Moreover, IL-6 has a positive correlation with HDRS and with YMRS and IL-2 has a positive correlation with YMRS. In addition, results showed that only IL-4 levels were increased in euthymic patients compared with healthy subjects. Our results are in accordance with previous studies suggesting that changes in proinflammatory cytokines may be associated with the pathophysiology of BD and pharmacological response (Kim et al., 2007; O'Brien et al., 2006). In fact, increased IL-6 has been one of the most consistent findings in BD (Knijff et al., 2007; Kim et al., 2007), but increased IL-4 and IL-2 have been less frequently described abnormalities (Ortíz-Domingues et al., 2007; Kim et al., 2007).

Importantly, there is only one study including a group of euthymic patients (Boufidou et al., 2004). The inclusion of this group can potentially offer data about the persistence or not of the proinflammatory state in remission. Demonstration of similar levels of the majority of proinflammatory cytokines between euthymic bipolar patients and control individuals could be an indication that inflammatory changes may probably be associated with acute mood episodes, especially acute mania. Increased IL-4 in euthymia must be carefully interpreted, because there was not a control of the time of

euthymia to the inclusion in this study. Therefore, it seems possible that cytokine levels have a slower return to baseline levels than mood symptoms.

This study was carefully conducted. The interview of cases and controls sample was performed using the same protocol, and this precaution was not taken in all other similar studies. In addition, the samples were collected between 8 and 9 a.m., minimizing circadian differences in cytokine levels that could potentially be related with cortisol release.

Limitations of cross-sectional studies preclude conclusions about causality. The use of medications by all bipolar participants could potentially be a limitation, but it is important to remember that mood stabilizers and antidepressants have predominantly anti-inflammatory action (Knijff et al., 2007; Ichiyama et al., 2000) and the differences alluded in this research might not be attributed to medication use. Another possible limitation in the present study is the measurement of cytokines in serum. Although previous studies have suggested that changes in peripheral levels may partly reflect the changes in brain cytokine secretion, there are no definitive conclusions about this issue (Pan and Kastin, 1999).

Studies comparing cytokines levels in medicated vs. unmedicated BD patients, and also before and after pharmacological treatment are warranted to control the potential confounders discussed here.

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The funders have no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

Dr. Kapczinski is supported by INCT, CNPq, CAPES, NARSAD and Stanley Medical Research Institute and is advisor and/or speaker for Janssen, Lilly, Abbott, Servier and AstraZeneca.

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Table 1. Characteristics of studied population

	Healthy subjects group	Patients with BD			*p*-value
		Euthymia	Mania	Depression	
N	25	14	23	24	–
Female gender (%)	80.0	71.4	52.2	62.5	0.22*
Age (mean ±S.D.)	43.4 (12.25)	44.2 (13.75)	40.8 (13.70)	45.0 (11.91)	0.72**
Caucasian (%)	83.3	85.2	83.3	75.0	0.28*
Years of schooling (mean±S.D.)	8.8 (4.63)	8.2 (5.12)	7.7 (2.90)	8.3 (4.19)	0.89**
Years of illness (median and interquartile range.)	–	21 (5–37)	7 (0–24)	26 (6– 36)	0,016 ***
HDRS score (mean±S.D.)	–	2.6 (1.91)	5.0 (2.34)	19.9 (9.35)	0.01**
YMRS score (mean±S.D.)	–	0.4 (0.63)	33.0 (9.15)	5.3 (4.17)	0.01**

HDRS: Hamilton Depression Rating Scale; YMRS: Young Mania Rating Scale.

SD: standart deviation.

* Chi square test

**One way ANOVA test

***Kruskall-Wallis test with post-hoc comparison.

Table 2. Cytokine profile in bipolar disorder (n=61) compared with healthy controls (n=25).

	Control group*	Patients with BD			*p*-value**
		Euthymia	Mania	Depression	
N	25	14	23	24	–
IL-2 (pg/ml)	0.00 (0.00–0.00)	0.00 (0.00–3.90)	3.20 (0.00–7.30)	0.00 (0.00–3.05)	0.016§
IL-4 (pg/ml)	0.00 (0.00–2.00)	2.10 (1.77–2.53)	1.90 (0.00–2.70)	1.90 (0.30–2.25)	0.020†, §
IL-6 (pg/ml)	0.00 (0.00–1.75)	0.75 (0.00–4.00)	3.30 (0.00–9.90)	2.20 (0.00–4.28)	0.008§. ‡
IL-10 (pg/ml)	0.00 (0.00–4.50)	3.05 (0.00–5.35)	2.80 (0.00–5.00)	0.90 (0.00–3.17)	0.430
IFNγ (pg/ml)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.995
TNFα (pg/ml)	0.00 (0.00–2.10)	0.00 (0.00–0.00)	0.00 (0.00–3.00)	0.00 (0.00–0.00)	0.342

BD:bipolar disorder; INF: interferon; TNF: tumor necrosis factor.

* Results in median and interquartile range.

** Kruskal-Wallis test.

Differences between groups assessed by Mann-Whitney test and Bonferroni's correction for three comparisons ($p \leq 0.007$), with euthymic versus controls p=0.007 (ǂ); manic versus controls: IL-2 p=0.001 (§), IL-4 p=0.011 (§), IL-6 p=0.002 (§) and depressed versus controls p=0.017 ‡.

Results described as 0.00 are below the detection limit of the test.

12 ARTIGO 3

Abnormalities in serum chemokine levels in euthymic patients with bipolar disorder

Brietzke E; Kauer-Sant'Anna M; Teixeira AL; Kapczinski F.

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ABNORMALITIES IN SERUM CHEMOKINE LEVELS IN EUTHYMIC PATIENTS WITH BIPOLAR DISORDER

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Abstract

The pathophysiology of bipolar disorder (BD) includes, among other processes, changes in the neuroplasticity and regulation of apoptosis, which could potentially be influenced by inflammatory mediators such as chemokines. The objectives of this study were to investigate serum chemokine levels in patients with BD and to compare results with those obtained with healthy subjects. Here, serum chemokine levels of euthymic patients with BD type I and 30 healthy volunteers were investigated and compared. The chemokines assessed were CCL2, CCL3, CCL8, CCL 9, CCL10, CCL11, and CCL24. Patients with BD showed significant differences in chemokine levels when compared with healthy subjects. While serum levels of CXCL10 were increased ($p = .018$), CCL24 levels were lower in bipolar patients ($p = .025$) when compared with controls. There was no statistical difference in the serum levels of CCL2, CCL3, CCL24, CXCL9, and CXCL11 between patients and controls. The presence of chemokine abnormalities in patients with BD during euthymia suggests that these inflammatory mediators should be further investigated with regard to their potential role as longstanding markers of the disorder.

1. Introduction

Although bipolar disorder (BD) is a highly prevalent and severe disorder, its pathophysiology has not yet been completely understood. Evidence has emerged showing an association of BD with changes in neuroplasticity and neuronal survival (Schloesser et al., 2008; Kapczinski et al., 2008a). These processes are influenced by several factors, including the orchestrated action of neurotransmitters, hormones, neurotrophins (Post, 2007; Kapczinski 53 et al., 2008b), and inflammatory mediators such as cytokines (Brietzke and Kapczinski, 2008). Recent evidence suggests the involvement of inflammatory cytokines in BD (Brietzke and Kapczinski, 2008; Kauer-Sant'anna et al., 2008; Soczynska et al., in press). Chemokines, a particular type of cytokines with chemoattractive properties, have received less attention in what concerns their role in BD. Nevertheless, these inflammatory mediators may be of particular interest in these patients, given their effect on the amplification of inflammatory response, possibly resulting in increased neuronal and glial death (Takahashi et al., 2008). For instance, peripheral chemokine levels seem to be altered in neurodegenerative disorders, such as multiple sclerosis (Moreira et al., 2006) and Alzheimer's disease (Galimberti et al., 2006; Reale et al., 2008; Kim et al., 2008), and in neuropsychiatric disorders such as schizophrenia (Drexhage et al., 2008; Teixeira et al., 2008) and major depressive disorder (Merendino et al., 2004; Sutcigil et al., 2007; Simon et al., 2008). Despite a growing body of evidence suggesting involvement of chemokines in psychiatric and neurodegenerative disorders, only one study so far has shown significantly increased levels of CXCL8 (IL-8) in patients with mania when compared with healthy subjects (O'Brien et al., 2006). To date, more than 50 chemokines and approximately 20 chemokine receptors – especially G-protein-coupled

receptors (Rostène et al., 2007) have been identified. The two largest families of chemokines, CCL and CXCL, attract mononuclear cells to sites of chronic inflammation. The binding of a chemokine to its receptor activates signaling cascades that lead to cell shape rearrange ment and movement (Charo and Ransohoff, 2006; Mackay, 2003; Murdoch and Finn, 2000). Activation of these signaling pathways results in increased calcium concentrations and activation of mitogen-activated protein kinases, and also has a role in synaptic plasticity (Rostène et al., 2007). For instance, CCL and CXCL chemokines have been shown to help in the prevention of neuronal apoptosis (Limatola et al., 2005; Ragozzino et al., 2006; Watson and Fan, 2005). Since the understanding of the inflammatory mechanisms involved in BD could potentially open new treatment possibilities (Brietzke and Kapczinski, 2008; Nery et al., 2008, the aim of this study was to investigate chemokine levels in BD patients compared with healthy subjects. Examples of the two largest families of chemokines were selected for assessment, namely CCL2 (MCP-1), CCL3 (MIP-1alpha), CXCL8 (IL-8), CXCL 9 (MIG-1), CXCL10 (IP-10), CCL11 (Eotaxin), and CCL24 (Eotaxin-2).

2. Methods

The present study was approved by the local ethics committee and all subjects provided written informed consent before inclusion in the study.

Thirty euthymic patients with BD type I were recruited from the outpatient clinic (Bipolar Disorders Program) at Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil. Psychiatric diagnosis was based on application of the Structured Clinical Interview for 105 DSM-IV-Axis I Disorders (SCID-I). Manic and depressive symptoms were assessed with the Young Mania Rating Scale (YMRS) and the Hamilton

Depression Rating Scale (HDRS), respectively. Patients were considered to be euthymic when DSM-IV criteria for mood episodes were not fulfilled and when HDRS and YMRS scores were below 7. Patients with any additional axis I or axis II DSM-IV diagnoses were excluded, as were those with general medical conditions associated with changes in inflammatory response, such as HIV/AIDS, rheumatologic diseases, current bacterial/viral disease, current use of more than 10 cigarettes/day, current use of corticosteroids or nonsteroidal anti-inflammatory medications, acetylsalicylic acid or immunosuppressives.

The healthy group was comprised of 30 volunteers who were screened for psychiatric disorders using the non-patient version of SCID-I. Subjects included in the study were not using any medication, and their first-degree relatives had no history of major psychiatric disorders, dementia, mental retardation, cancer, or tumor. Exclusion criteria for healthy subjects were the same as those used in the selection of euthymic patients.

Five milliliters of blood were collected from each subject by venipuncture in anticoagulant-free vacuum tubes. Samples were immediately centrifuged at 3000g for 5 min, and the serum was kept frozen at _80 _C until assayed.

The serum concentration of chemokines was determined using sandwich ELISA kits, following the manufacturer's protocol (DuoSet R&D Systems, Minneapolis, MN, USA). For the analysis, samples were thawed and excess proteins removed by acid/salt precipitation, as routinely performed in our laboratory (Moreira et al., 2006; Teixeira et al., 2008). Briefly, an equal volume of serum and 1.2% trifluoroacetic acid/1.35 M NaCl were mixed and left at room temperature for 10 min. Samples were then centrifuged for

136 5 min at 3000g, and the supernatants adjusted for salt content (0.14 M sodium chloride and 0.01 M sodium phosphate) and pH (7.4) for the determination of chemokine levels. All samples were assayed in duplicate. Assay detection limits were 5 pg/ml.

2.1. Statistics

Descriptive analyses included assessment of the distribution of all variables; data are presented as means and percentages. Demographic and clinical characteristics were analyzed using the v2-test and analysis of variance (ANOVA), as indicated. Chemokine levels showed a non-parametric distribution and were analyzed with appropriated tests, as indicated. Differences between the two groups assessed were evaluated using the Mann–Whitney U test.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA). In view of the non-normal distribution observed, effect size (ES) measurements were determined with a logarithmic transformation. Statistical significance was set at $p < 0.05$.

3. Results

Demographic and clinical characteristics of the sample (n = 60) are presented in Table 1. Both groups were homogeneous in terms of age, sex, ethnic group and years of schooling. Median and 25th and 75th percentiles for chemokine levels in BD patients and healthy subjects are presented in Table 2. Analysis of serum chemokine levels revealed significantly higher levels of CXCL10 (IP-10) (U = 291.5, p = 0.018, Z = _2.369, ES = _0.71) and lower levels of CCL24 (Eotaxin-2) (U = 287.0, p = 0.025, Z =

_2.244, ES = _0.63) in BD patients when compared with healthy subjects; no differences were observed in the other chemokines assessed (Table 2). There was no correlation between chemokine levels and duration of disorder (years of illness).

4. Discussion

Our results revealed an increase in inflammatory chemokine CXCL10 and a reduction in CCL24 levels in euthymic BD patients when compared with healthy control subjects, suggesting an association between BD and changes in inflammatory status. Such changes are persistent during interepisodic periods and involve chemokines. Whether inflammatory abnormalities in BD persist or not in the remission of mood episodes is an important research question that has been scarcely investigated so far. Our results are in accordance with previous studies which suggest that changes in inflammatory mediators may be associated with the pathophysiology and pharmacological response of BD (Kim et al., 2007; O'Brien et al., 2006). In addition, evidence that chemokines play an important role in synaptic plasticity has been reported. CCL5, CCL22, CX3CL1, and CXCL12 have been shown to protect hippocampal neurons from amyloid-beta-peptide-induced neurotoxicity (Watson and Fan, 2005), and CXCL12 has demonstrated a potential to protect rat cerebellar neurons from apoptosis (Limatola et al., 2000). The presence of chemokine abnormalities in euthymic patients indicates that these inflammatory mediators could be longstanding markers of the disease.

Increased levels of the chemokine CXCL10, a Th1-related mediator, and, at the same time, decreased levels of Th2-related hypersensitivity-related CCL24 were found. These findings are suggestive of imbalance of Th1/Th2 cytokines rather than a simple

increase in cytokines and these changes are similar to those described in a number of inflammatory conditions and also in depressive disorder (Huang and Lee, 2007; Myint et al., 2005; Gabbay et al., 2009) and schizophrenia (Kim et al., 2004). Interestingly antidepressant could potentially correct this imbalance (Myint et al., 2005).

Regardless of the etiology of chronic inflammatory abnormalities in individuals with mood disorders, it is known that they may result in downstream biological damage and contribute to elevated cardiac and other medical morbidities associated with BD (McE wen, 2003; Simon et al., 2008; Kapczinski et al., 2008b). In addition, there is strong evidence of the association between untreated BD and increased rates of death from cardiovascular and cerebrovascular events, a finding that gives a systemic dimension to BD (Angst et al., 2002; Kupfer, 2005). One of the links between mood disorders and general medical diseases could be the increase of proinflammatory mediators, which are recognized causal factors in the develop ment and progression of arteriosclerosis (Leitinger, 2008).

Several questions remain to be answered. One of the most important questions is related to what happens with chemokines levels during the follow-up of remissions. It is possible that mood symptoms remit before the normalization of inflammatory mediators. In addition, current knowledge is limited in what concerns the physiological role of chemokines in the central nervous system and the specific mechanisms of regulation and balance of different inflammatory mediators in conditions of health and disease.

This study has strengths and limitations that should be considered for the interpretation of results. The diagnostic interviews of both patients and controls were performed using the same protocol, overcoming a limitation of previous similar studies.

In addition, blood samples were withdrawn between 8 and 9 a.m., minimizing circadian differences in cytokine levels potentially related with cortisol release. On the other hand, limitations inherent to cross-sectional studies (and thus also present here) preclude conclusions about causality. The use of medications by all BD patients could potentially be a limitation; however, evidence suggests that mood stabilizers and antidepressants have predominantly an anti-inflammatory action (Knijff et al., 2007; Ichiyama et al., 2000), and therefore the differences herein observed cannot be fully attributed to medication use. Another possible limitation is that chemokines were measured in serum. Although previous studies have suggested that changes in peripheral levels may partly reflect changes in brain cytokine secretion, there are no definitive conclusions about this issue (Pan and Kastin, 1999).

In sum, our study is significant in that it is the first to provide evidence of the involvement of such chemokine abnormalities in BD. If the present findings are corroborated by future reports, they could possibly open a new path in the field of BD treatment options and in neuroscience research as a whole, contributing to the understanding of the complex and delicate balance between neuronal life and death. Such a complexity should be taken into account in the development of drugs that exert their effects through chemokines and that might have a therapeutic use in BD (Rostène et al., 2007).

Conflict of interest

Dr. Kapczinski is supported by INCT, CNPq, CAPES, NARSAD and Stanley Medical Research Institute and is advisor and/or speaker for Janssen, Lilly, Abbot, Servier and Astra Zeneca.

Dr. Kauer-Santanna has been investigator of clinical trials sponsored by CNPq, Canadian Institute of Health Research and Stanley Foundation, has received salary support from an APA/Astra-Zeneca unrestricted educational grant and is a NARSAD young investigator.

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Table 1.

10 CONCLUSÃO

Os resultados dos artigos que compõem esta tese indicam que, no TB, existe uma ativação da resposta inflamatória, evidenciada pela elevação de citocinas pró-inflamatórias no plasma. As causas dessa elevação ainda não podem ser estabelecidas, mas podem ter diversas origens, dentre elas a genética e os eventos ambientais adversos.

A morte celular e a atrofia que ocorrem no TB podem ser conceitualizadas como originadas a partir de um prejuízo na resiliência celular. McEwan (2000), elegantemente, elaborou o conceito de carga alostática (1,2), posteriormente aplicado ao TB (3). Muitos fatores que são fundamentais para a sobrevida podem, ao longo do tempo, exigir do organismo um custo que acelera os processos patológicos (carga alostática). Existem indícios de que a atrofia regional no TB pode ocorrer devido à magnitude e à duração das alterações bioquímicas e também imunológicas que acompanham os episódios da doença. Assim, um excesso de atividade pró-inflamatória se constituiria em uma importante contribuinte para o aumento da carga alostática no TB.

10.1 Implicações clínicas dos achados dos artigos

10.1.1 Participação das citocinas inflamatórias como marcador de toxicidade sistêmica no TB

Algumas citocinas, como o TNF-alfa, a IL-6 e a IL-10, quando usadas em associação com marcadores de estresse oxidativo e com a redução de neurotrofinas, mostraram-se úteis na mensuração da toxicidade sistêmica dos episódios de humor.

Quando comparados a pacientes com sepse, os pacientes em mania e em depressão bipolar mostraram menor toxicidade sistêmica, mas, mesmo assim, com valores elevados em relação a bipolares eutímicos e controles (4).

10.1.2 Possível associação dos marcadores inflamatórios com achados de neuroimagem

A despeito do debate considerável a respeito das anormalidades estruturais no TB, uma meta-análise recente confirmou a existência de alargamento dos ventrículos laterais e hiperintensidades de substância branca no TB (5). Novos métodos de neuroimagem, como a *diffusion tensor imaging* (DTI), tem se mostrado capazes de revelar detalhes da substância branca (6). As anormalidades detectadas por esse método refletem modificações gliais e alterações na citoarquitetura que são consistentes com alterações na conectividade cortical-subcortical (6). Estudos com doenças infecciosas cerebrais e esclerose múltipla revelaram que alterações no DTI correspondem a lesões causadas por processos inflamatórios no SNC (6,7,8,9). Estudos futuros são necessários para avaliar a associação entre citocinas inflamatórias e achados relacionados com o DTI.

10.1.3 Possibilidades terapêuticas do controle da atividade inflamatória no TB

10.1.3.1 Importância do controle do uso de álcool e tabaco

O TB e os transtornos por uso de substância frequentemente ocorrem em comorbidade (10) e são associados com morbidade e mortalidade significativas (11). O

álcool tem propriedades pró-apoptóticas neuronais bem demonstradas (12), induz a produção de espécies reativas de oxigênio e interage com vias envolvidas na resposta celular a citocinas (13). O uso crônico de álcool estimula as células gliais a aumentarem a produção e expressão de mediadores inflamatórios no cérebro e ativa vias de sinalização que culminam na transcrição de fatores envolvidos no dano inflamatório aos neurônios e glia (14).

De maneira similar, o uso de nicotina é muito mais comum entre pacientes bipolares do que na população em geral, com uma *odds ratio* de 5,0 para uso atual de tabaco, de 2,6 para uso na vida e de 0,13 para cessação do fumo (15). O uso de cigarro é associado com idade precoce de início, maior gravidade dos sintomas, funcionamento pobre, história de tentativa de suicídio e comorbidade com transtornos de ansiedade e transtornos por uso de substância (16). Pessoas que fumam têm aumento de citocinas inflamatórias no sangue, como IL-1, IL-8, INF-gama e TNF-alfa (17-22).

Considerando-se os efeitos deletérios do álcool e do tabaco sobre a inflamação, esse mecanismo pode, potencialmente, estar implicado no efeito deletério do uso dessas substâncias, e a cessação do seu uso pode impactar o curso do TB mediante efeitos imunomodulatórios.

10.1.3.2 Medicações anti-inflamatórias como adjuvante a medicações psiquiátricas

O papel potencial dos agentes anti-inflamatórios como adjuvantes no tratamento do TB tem sido foco de alguns estudos recentes. Um ensaio clínico randomizado contra placebo com o inibidor da Cox-2 celecoxib mostrou que os pacientes que receberam esta medicação tinham redução significativa dos escores de depressão após a primeira

semana de tratamento em comparação aos que receberam placebo (23). Levando em consideração o resultado desses estudos juntamente com os que já foram realizados na depressão maior e na esquizofrenia, o celecoxib emerge como uma alternativa promissora de neuromodulação no TB (24).

Outra possível alternativa é o uso de inibidores de TNF-alfa (adalimumab, infliximab e etanercept), atualmente usados para o tatamento de diversas doenças autoimunes. Ensaios clínicos com essa classe de drogas em pacientes com doença de Crohn (25,26,27) e psoríase mostraram redução de sintomas depressivos (28,29,30). Além disso, existem evidências preliminares de que o etanercept pode produzir melhora cognitiva na doença de Alzheimer (31). Esses resultados suportam a realização de ensaios específicos com essa medicação no TB.

10.2 Perspectivas

O cérebro é uma região de funcionamento extremamente complexo, que interage com o sistema imune onde este desenvolve funções de proteção, mas também de neuromodulação, conectividade e regulação da apoptose de neurônios e glia. Os resultados dos estudos que compõem esta tese corroboram a literatura existente até o momento no que diz respeito à associação de TB com citocinas inflamatórias, embora detalhes dessa associação não sejam conhecidos.

A inflamação oferece, também, um novo campo de investigação para a terapêutica do TB e também para a elucidação da associação entre TB e comorbidades clínicas. Com o entendimento dos mecanismos da toxicidade sistêmica no TB, o foco do

tratamento necesssariamente deve mudar do controle sintomático para o controle das anormalidades neuropatológicas e periféricas.

Além disso, determinar o ponto exato em que a inflamação deixa de ser o "mocinho" e se transforma em "vilão" oferece importantes questões de pesquisa para a genética, epigenética, neuropsicologia, neuroimagem e psicofarmacologia. Os mecanismos moleculares envolvidos na resposta imune devem, necessariamente, ser elucidados e traduzidos em tratamentos inovadores que possam produzir mudanças de desfechos no TB.

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Anexo 1. Termo de Consentimento Livre e Esclarecido para pacientes:

MEDIADORES INFLAMATÓRIOS NO TRANSTORNO BIPOLAR

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO:

Nome:

Datade Nascimento:

Médico Supervisor: Flávio Kapczinski

Antes de participar deste estudo, gostaríamos que você tomasse conhecimento do que ele envolve. Damos abaixo alguns esclarecimentos sobre dúvidas que você possa ter. Em caso de qualquer outra dúvida quanto ao estudo que ele envolve e sobre os seus direitos, você deverá contatar a médica Elisa Brietzke pelo telefone (51) 81375044, de segunda a sexta-feira, das 9 às 18 horas ou contactá-la pessoalmente no Ambulatório do PROTAHBI (zona 12) às sextas-feiras das 16 às 19 horas.

Qual o objetivo desta pesquisa?

Conhecer características clínicas dos pacientes e sua possível relação com alterações existentes no sangue (alterações imunológicas). Existe uma possibilidade de associação dessas alterações imunológicas com o transtorno bipolar, mas mais estudos devem ser feitos para constatar tal afirmação. Ou seja, este estudo tem por objetivo, de uma forma geral, conhecer um pouco melhor como "funciona" o transtorno bipolar.

O que acontecerá neste estudo?

Após os pacientes e familiares responderem às questões em estudo através de questionários será feito um exame de sangue. Este exame será utilizado apenas para observar estas alterações.

Quais os benefícios em participar deste estudo?

A sua participação neste estudo poderá proporcionar, no âmbito pessoal, a identificação de algum problema que não fosse antes conhecido, e no âmbito coletivo, poderá ajudar no desenvolvimento de maior conhecimento do transtorno bipolar principalmente em relação às causas da doença e como ela funciona.

Quais são os direitos dos participantes?

Os pesquisadores deste estudo podem precisar examinar os seus registros, a fim de verificar as informações que fazem parte do objetivo deste estudo. No entanto, os seus registros médicos serão sempre tratados confidencialmente. Os resultados deste estudo poderão ser enviados para publicação em um jornal científico, mas você não será identificado por nome em nenhum momento. Sua participação no estudo é voluntária, de forma que, caso você decida não participar, esta decisão não afetará o tratamento normal ao que o paciente tem direito.

Quais são os riscos que envolvem este estudo?

Este estudo possui riscos mínimos que são inerentes do procedimento de punção venosa.

Quais são as responsabilidades dos participantes?

Os participantes deste estudo comprometem-se a responder fidedignamente as escalas aplicadas pelos médicos pesquisadores e as escalas auto-aplicáveis.

Declaro que:

1. Recebi uma explicação completa do objetivo do estudo, dos procedimentos envolvidos e o que se espera minha pessoa.

2. Estou ciente de que tenho total liberdade de desistir do estudo a qualquer momento e que esta desistência não irá, de forma alguma, afetar meu tratamento médico atual ou futuro na instituição.

3. Estou ciente de que a informação nos meus registros médicos é essencial para a avaliação dos resultados estudo. Concordo em liberar esta informação sob o entendimento de que ela será tratada confidencialme ou seja, não serei referido por nome em qualquer relatório relacionado a este estudo.

4. Concordo total e voluntariamente em fazer parte deste estudo; tenho mais de 18 anos.

Assinatura do Paciente	Assinatura do Médico Supervisor
Ass:	Ass:
Data:	Data:

Assinatura do Familiar
Responsável pelo Paciente

Ass:

Data:

Anexo 2. Termo de Consentimento Livre e Esclarecido para controles.

MEDIADORES INFLAMATÓRIOS NO TRANSTORNO BIPOLAR

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO PARA VOLUNTÁRIOS:

Nome:

Data de Nascimento:

Médico Supervisor: Flávio Kapczinski

Esta é uma pesquisa para identificar substâncias que podem estar alteradas no sangue de indivíduos portadores de Transtorno Bipolar (alterações imunológicas). Nesta pesquisa os voluntários serão convidados a participar através da coleta uma amostra de sangue e do preenchimento de algumas questões em questionários. O sangue coletado será armazenado e algumas substâncias contidas nele serão avaliadas em conjunto com alguns dados coletados de pacientes portadores de Transtorno Bipolar.

Esta pesquisa é considerada como de risco mínimo para a saúde, podendo ocorrer apenas um mal-estar passageiro ou manchas roxas no local da coleta de sangue. Seu nome e seus dados serão mantidos confidenciais pelos pesquisadores, sendo estes dados utilizados somente para pesquisa.

Eu, _____, fui informado(a) dos objetivos especificados acima e da justificativa desta pesquisa, de forma clara e detalhada. Recebi informações específicas sobre cada procedimento no qual estarei envolvido, dos desconfortos ou riscos previstos, tanto quanto benefícios esperados. Todas as minhas dúvidas foram respondidas com clareza e sei que poderei solicitar novos esclarecimentos a qualquer momento. Além disso, sei que terei liberdade de retirar meu consentimento para a participação na pesquisa de acordo com essas informações, sem que isto me traga prejuízos.

Assinatura do voluntário Assinatura do pesquisador

Local e data

Em caso de qualquer outra dúvida quanto ao estudo e o que ele envolve e sobre os seus direitos, você deve contatar a médica Elisa Brietzke pelo telefone (051)81375044 de segunda a sexta-feira das 9 às 18 horas.

Anexo 3. Protocolo de Pesquisa

PROTOCOLO DE PESQUISA

Protocolo no.

Data do preenchimento:

Entrevistador:

1. **Identificação**

1.1. Prontuário:

1.2. Nome Completo:

1.3 Sexo: (1) masculino (2) feminino

1.4 Data de Nascimento:

1.5 Idade: anos

1.6 Etnia: (1) branco (2) não-branco

1.7 Situação Conjugal: (1) solteiro (2) casado ou companheiro fixo
(3) separado ou divorciado (4) viúvo

1.8. Ocupação: (1) estudante (2) com ocupação (remunerada)

(3) sem ocupação (não aposentado) (4) dona de casa

(5) em auxílio-doença (6) aposentado por invalidez

(7) aposentado por tempo de serviço

1.9 Renda Individual: (1) sim (2) não

1.10 Renda Familiar: R$ ----------(US$) ----------

1.11. Escolaridade: ---------- Grau (1) completo (2) incompleto

(----------) anos de estudo completos

1.12. Repetência escolar: ---------- anos repetidos

1.13.Sabe ler e escrever? (1) sim (2) não

1.14. Endereço:

1.15. Cidade: CEP:

1.16. Telefone para contato: (1) ----------

(2) ----------

1.17- Peso atual (medido): ---------- kg

1.18- Altura (medida): ---------- m

2. Características da Doença

2.1. Idade do 1o episódio:---------- anos

2.2. Tempo de evolução da doença: ---------- anos

2.3. Tipo do 1o episódio (DSM-IV): (1) Mania (2) Depressão

(3) Misto (4) Hipomania

(9) Não sabe

2.4. Idade em que recebeu diagnóstico médico: ---------- anos

2.5. Idade em que usou medicação psiquiátrica com indicação médica pela primeira vez:

anos. Qual?

2.6. Idade em que usou estabilizador de humor pela primeira vez: anos.

2.7. Primeiro episódio desencadeado por substância? (1) sim (2) não

2.8.Se sim: (1) antidepressivo. Qual? ----------

(2) maconha

(3) cocaína

(4) álcool (2) Depressão (3) Misto

(9) Não sabe

(5) anorexígeno. Qual? ----------

(6) estimulantes

(7) Hormônios. Qual?----------

(8) Outros. Qual? ----------

2.9. Hospitalizações Psiquiátricas: (1) sim (2) não

Se sim, quantas: ----------

2.9. Idade na 1a hospitalização psiquiátrica: anos

Tipo de episódio (DSM-IV): (1) Mania

(4) Hipomania

2.10. Idade na última hospitalização: anos

Tipo de episódio (DSM-IV): (1) Mania (2) Depressão (3) Misto

(4) Hipomania (9) Não sabe

2.11. Ciclador rápido (4 ou mais episódios em um ano): (1) sim (2) não

2.12. Realizou ECT: (1) sim (2) não

Se sim, quantas séries? ----------

2.13. Tentativa de Suicídio: (1) sim (2) não

Se sim, número:---------- vezes

Método: (1) arma de fogo (2) enforcamento (3) cortar os pulsos

(4) medicação (5) pular de local alto (6) outro. ----------

3. Hábitos

3.1. Tabagismo passado: (1) sim (2)não Se sim, tempo de uso: ----------anos

3.2. Tabagismo atual: (1) sim (2)não Se sim, maior no de cigarros/dia: ----------

3.3 Uso atual de: Chá (1) sim (2)não

Chimarrão (1) sim (2)não

Café (1) sim (2)não

4. Tratamento Psiquiátrico

4.1. Você já foi submetido a algum tratamento psiquiátrico? (1) sim (2) não

Quais medicamentos você já utilizou?

Nome da medicação	Já usou alguma vez?		Uso atual		Dose atual (total/dia)
Alprazolan (Frontal)	Sim	não	sim	não	
Ácido valpróico (Depakene)	Sim	não	sim	não	
Amitriptilina (Tryptanol)	Sim	não	sim	não	
Biperideno (Akineton)	Sim	não	sim	não	
Bupropiona (Zyban)	Sim	não	sim	não	
Carbamazepina (Tegretol)	Sim	não	sim	não	
Citalopram (Cipramil)	Sim	não	sim	não	
Clomipramina (Anafranil)	Sim	não	sim	não	
Clonazepan (Rivotril)	Sim	não	sim	não	
Clonidina (Atensina)	Sim	não	sim	não	
Clorpromazina (Amplictil)	Sim	não	sim	não	
Clozapina (Leponex)	Sim	não	sim	não	
Diazepam (Valium)	sim	não	sim	não	
Escitalopram (Lexapro)	sim	não	sim	não	
Fluoxetina (Prozac)	sim	não	sim	não	
Haloperidol (Haldol)	sim	não	sim	não	
Imipramina (Tofranil)	sim	não	sim	não	
Lítio (Carbolitium)	sim	não	sim	não	
Levomepromazina (Neozine)	sim	não	sim	não	
Lamotrigina (Lamictal)	sim	não	sim	não	
Metilfenidato (Ritalina)	sim	não	sim	não	
Mirtazapina (Remeron)	sim	não	sim	não	
Olanzapina (Zyprexa)	sim	não	sim	não	
Paroxetina (Aropax)	sim	não	sim	não	
Pimozida (Orap)	sim	não	sim	não	
Quetiapina (Seroquel)	sim	não	sim	não	
Risperidona (Risperdal)	sim	não	sim	não	
Sertralina (Zoloft)	sim	não	sim	não	
Sulpiride (Equilid)	sim	não	sim	não	
Venlafaxina (Efexor)	sim	não	sim	não	
Outras:	sim	não	sim	não	
	sim	não	sim	não	

5. História Familiar de Doença Psiquiátrica

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(1) Bip V: com diagnóstico de TB validado

(2) Uso de Lítio

(3) Alucinações/ "ficou louco"

(4) Tentativa de Suicídio

(5) Internações Psiquiátricas

(6) Uso de antipsicótico

(7) Suicídio

(8)Álcool e drogas

Pai				Transtorno Psiquiátrico
Mãe				
Pai				
Irmãos. N----------				
Filhos: N ----------				
Avó materna				
Avô materno				
Avó paterna				
Avô paterno				
Tios maternos				
Tios paternos				
Primos maternos				
Primos Paternos				

5.1. No de familiares de 1o grau acometidos por Bip V: membros

5.2. No de familiares de 1o grau acometidos por outras doenças psiquiátricas: membros.

6. Fatores relacionados ao sexo feminino

6.1- Uso de método anticoncepcional: (1) sim (2) não Se sim, qual(is):

(1) Anticoncepcional oral (pílula)

(2) DIU

(3) Preservativo

(4) Diafragma

(5) Coito interrompido

(6) Ligadura Tubária

(7) Vasectomia

(8) Tabelinha

(9) outro. Qual ----------

6.2. Gestações: ----------

6.3. Partos Normais: ----------

6.4. Cesarianas: ----------

6.5. Abortos: ---------- espontâneos; ---------- provocados

6.6. Menopausa: (1) sim (2) não

6.7. Terapia de Reposição Hormonal (atual): (1) sim (2) não

7. História de Doenças Clínicas (atual e passada)

7.1 Você já teve alguma dessas doenças?

1	sim	não	não sei	Quando	Infecção do SNC (Poliomilelite, encefalite,
2	sim	não	não sei	Quando	Infecção freqüente na garganta
3	sim	não	não sei	Quando	Coma
4	sim	não	não sei	Quando	Convulsão/ "ataque"epilético
5	sim	não	não sei	Quando	Ferimento na cabeça (com perda de
6	sim	não	não sei	Quando	Enxaqueca
7	sim	não	não sei	Quando	Asma
8	sim	não	não sei	Quando	Diabetes
9	sim	não	não sei	Quando	Hipotireoidismo
10	sim	não	não sei	Quando	Hipertireoidismo
11	sim	não	não sei	Quando	Febre reumática
12	sim	não	não sei	Quando	Derrame Cerebral
13	sim	não	não sei	Quando	
14	sim	não	não sei	Quando	
	sim	não	não sei	Quando	

Se sim para qualquer dos itens acima:

Quais doenças foram diagnosticadas por um médico? ----------

Você é portador de alguma doença não perguntada? ----------

8. Desenvolvimento

8.1. Qual a idade da sua mãe quando você nasceu? ----------

8.2. Existiu alguma intercorrência durante a sua gestação?

(1) sim (2) não (3) não sei

Durante a sua gestação, sua mãe fez uso de:

8.3. Medicações? (1) sim (2) não (3) não sei

Se sim, quais? ----------

8.4. Drogas? (1) sim (2) não (3) não sei

Se sim, quais? ----------

8.5. Cigarro? (1) sim (2) não

8.6. Álcool? (1) sim (2) não

8.7. Seu parto foi: (1) normal (2) cesariana

8.8. Prematuridade

(1) sim (2) não

8.9. Circular de cordão:

(1) sim (2) não

8.10. Uso de fórcipe:

(1) sim (2) não

8.11. Você teve alguma complicação após o nascimento, necessitando hospitalização? (1) sim (2) não (3) não sei

8.12. Você teve algum atraso no desenvolvimento (sentar, caminhar, falar)?

(1) sim (2) não (3) não sei