UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO

RAYANE BERNARDES ESTEVAM

ANÁLISE DA EXPRESSÃO GÊNICA *IN SITU* DE mRNA PARA MOLÉCULAS MODULADORAS DA RESPOSTA IMUNE E DA INFLAMAÇÃO NA PSORÍASE EM PLACA.

UBERABA-MG

2022

RAYANE BERNARDES ESTEVAM

ANÁLISE DA EXPRESSÃO GÊNICA *IN SITU* DE mRNA PARA MOLÉCULAS MODULADORAS DA RESPOSTA IMUNE E DA INFLAMAÇÃO NA PSORÍASE EM PLACA.

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde, área de concentração Imunologia Básica e Aplicada, da Universidade Federal do Triângulo Mineiro, como requisito parcial para obtenção do título de Doutora em Imunologia Básica e Aplicada.

Orientador(a): Prof. Dra. Denise Bertulucci Rocha Rodrigues.

Coorientador: Prof. Dr. Virmondes Rodrigues Júnior.

UBERABA-MG
2022

Catalogação na fonte: Biblioteca da Universidade Federal do Triângulo Mineiro

E84a

Estevam, Rayane Bernardes
Análise da expressão gênica *in situ* de mRNA para moléculas moduladoras da resposta imune e da inflamação na psoríase em placa / Rayane Bernardes Estevam. -- 2022.
83 f. : graf., tab.

Tese (Doutorado em Ciências da Saúde) -- Universidade Federal do Triângulo Mineiro, Uberaba, MG, 2022
Orientadora: Profa. Dra. Denise Bertulucci Rocha Rodrigues
Coorientador: Prof. Dr. Virmondes Rodrigues Júnior

1. Psoríase. 2. Citocinas. 3. Arginase. 4. Metotrexato. I. Rodrigues, Denise Bertulucci Rocha. II. Universidade Federal do Triângulo Mineiro. III. Título.

CDU 616.517

Mirtes Soares - Bibliotecária CRB-6/2181

À Deus, pois é Dele que recebi toda sabedoria, é Ele Quem guia todos os meus passos.

A toda minha família...

Minha mãe Maria Aparecida com suas orações e amor incondicional me fortaleciam todos os dias.

Meus irmãos Milla e Paulo Henrique, que sempre me incentivaram para alcançar meus sonhos, em especial a este, que tanto batalhei, e hoje se torna realidade.

A meu esposo Matheus pelo amor, compreensão e apoio constante na conquista deste sonho.

A meu pai Paulo (in memoriam) que está presente a todo o momento no meu coração... Uma imensa saudade, mas a certeza de seus aplausos pela minha conquista.

AGRADECIMENTOS

- ✓ Primeiramente a Deus, pela dádiva da vida. Por caminhar ao meu lado, e diariamente me ajuda a enfrentar os obstáculos, e comigo celebra e compartilha as alegrias da minha vida.
- ✓ À minha orientadora Profª Dra. Denise Bertulucci Rocha Rodrigues, pelos ensinamentos, incentivo e confiança em tornar possível esta conquista. Levarei como referência de profissionalismo.
- ✓ Ao Prof. Dr. Virmondes Rodrigues que com a sua experiência e competência colaborou com a conclusão deste estudo.
- ✓ As amigas da pós-graduação Danila Malheiros e Isabela Rios, pela amizade que construímos ao logo do mestrado e doutorado, apoiando uma as outras trançando ao mesmo objetivo rumo ao sucesso.
- ✓ A Bethânia, funcionária do Laboratório de Imunologia pela disponibilidade, paciência e auxílio na parte prática deste trabalho.
- ✓ As amigas da UNIMED Uberaba pela amizade e apoio, sempre me incentivando a esta conquista.
- ✓ A aluna de mestrado Laís Milagres tão prestativa e dedicada nos auxiliou no fechamento do trabalho.
- ✓ Aos amigos do laboratório Jonatas, Chamberttan, pelos ensinamentos, horas de estudo e colaboração do trabalho.
- ✓ Ao Dr. Sandro Furiati, pela confiança em disponibilizar as biópsias dos pacientes com psoríase do estudo.
- ✓ Aos professores da pós-graduação, pelo empenho em cultivar o incentivo pela pesquisa e docência.
- ✓ Aos meus queridos amigos, que a cada conquista brindavam comigo, que nos obstáculos me ajudavam a me erguer, por entenderem minha ausência em tantos eventos especiais. Sou grata por ser abraçada por tantas pessoas boas.
- ✓ Enfim, um sincero agradecimento a todos que colaboraram para a realização deste trabalho.

Obrigada por torcerem por mim, por minha FELICIDADE e por meu SONHO!

"Que os vossos esforços desafiem as impossibilidades, lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível."

Charles Chaplin

RESUMO

A psoríase é uma doença inflamatória crônica, caracterizada por hiperplasia epidérmica. A fisiopatologia da doença é caracterizada pela interação entre sistema imune inato e adaptativo, mediada pela ativação inapropriada de linfócitos T, do qual estão associados a uma complexa rede de células e mediadores inflamatórios atuando sinergicamente na indução e manutenção do processo inflamatório. A produção exacerbada das moléculas envolvidas em sua patogenia induz a hiperproliferação dos queratinócitos, fazendo com que estes também produzam alguns fatores inflamatórios e precursores da inflamação, potencialmente envolvidos na patogênese da psoríase. Diante disto, o presente estudo avaliou a expressão gênica *in situ* de mRNA de moléculas moduladoras da resposta imune e da inflamação na psoríase em placa. Analisou também a expressão gênica destas moléculas em pacientes com psoríase em terapia com metotrexato. A casuística foi constituída por 26 pacientes, destes, 21 foram diagnosticados com psoríase moderada a grave (PASI> 10). Foram coletadas biópsias de duas regiões distintas do mesmo paciente, uma diretamente do tecido lesional e outra do tecido não lesional. Destes, 15 estavam em uso da terapia com metotrexato e 6 estavam sem uso de medicação. Como grupo controle foram selecionados 5 pacientes saudáveis. Para análise da expressão gênica *in situ,* o material foi processado para extração do mRNA, confecção de cDNA e finalmente realização de qPCR dos genes *IL-17, IL-23, IL-22, IL-10, IFN-γ, TNF-α, TGF-βR2, ARG-1, ARG-2, iNOS e GLUT-1*. Os resultados mostraram que a expressão gênica *in situ* de todos os genes avaliados foi significativamente maior nas amostras de tecido lesional e não lesional dos pacientes com psoríase do que no tecido dos indivíduos saudáveis. A expressão dos genes *IL-17, IFN-γ,* da ARG-1 e ARG-2, foram significativamente maiores no tecido lesional comparado com o tecido não lesional. A terapia com metotrexato aumenta a expressão de mRNA do IFN-γ, da IL-10, do TGF-βR2 e da ARG-2, em pacientes com psoríase em placa. A superexpressão destas moléculas em tecido lesional e não lesional parecem atuar no estado patológico da hiperproliferação epidérmica e na manutenção do processo inflamatório relevante na imunopatogêse da psoríase em placa.

Palavras-chave: Psoríase; Citocinas; Arginase; GLUT e Metotrexato.

ABSTRACT

Psoriasis is a chronic inflammatory disease characterized by epidermal hyperplasia. The pathophysiology of the disease is characterized by the interaction between the innate and adaptive immune system, mediated by the inappropriate activation of T lymphocytes, which are associated with a complex network of cells and inflammatory mediators acting synergistically in the induction and maintenance of the inflammatory process. The exacerbated production of molecules involved in its pathogenesis induces hyperproliferation of keratinocytes, causing them to also produce some inflammatory factors and precursors of inflammation, potentially involved in the pathogenesis of psoriasis. In the face of this, the present study evaluated the *in situ* gene expression of mRNA of molecules that modulate the immune response and inflammation in plaque psoriasis. He also analyzed the gene expression of these molecules in patients with psoriasis on methotrexate therapy. The series consisted of 26 patients, of whom 21 were diagnosed with moderate to severe psoriasis (PASI> 10). Biopsies were collected from two different regions of the same patient, one directly from the lesional tissue and the other from non-lesional tissue. Of these, 15 were using methotrexate therapy and 6 were not using medication. As a control group, 5 healthy patients were selected. For *in situ* gene expression analysis, the material was processed for mRNA extraction, cDNA production, and finally qPCR of *IL-17, IL-23, IL-22, IL-10, IFN-γ, TNF-α* genes, *TGF-βR2*, ARG-1, ARG-2, iNOS, and GLUT-1. The results showed that the *in situ* gene expression of all evaluated genes was significantly higher in lesional and non-lesional tissue samples from patients with psoriasis than in tissue from healthy individuals. Expression of IL-17, IFN-γ, ARG-1 and ARG-2 genes was significantly higher in lesional tissue compared to non-lesional tissue. Methotrexate therapy increases IFN-γ, IL-10, TGF-βR2, and ARG-2 mRNA expression in patients with plaque psoriasis. The overexpression of these molecules in lesional and non-lesional tissue seems to act in the pathological state of epidermal hyperproliferation and the maintenance of the relevant inflammatory process in the immunopathogenesis of plaque psoriasis.

Keywords: Psoriasis; Cytokines; Arginase; GLUT and Methotrexate.

LISTA DE ABREVIATURAS

PASI: Psoriasis Area Severity Index

TCLE: Termo de Consentimento Livre e Esclarecido

PSORS: Psoriasis Susceptibility

GWAS: Associação Ampla de Genoma

MTX: Metotrexato

MHC: Complexo Principal de Histocompatibilidade

HLA: Antígeno Leocucitário Humano

APC: Célula apresentado de antígeno

DC: Célula dentrítica

ICAM: Molécula de adesão Intracelular

CD4: Linfócito T auxiliares

CD8: Linfócito T citolíticos

NO: Óxido Nítrico

Th1: Subtipo de Linfócito auxiliar do perfil 1

Th2: Subtipo de Linfócito auxiliar do perfil 2

Treg: Linfócitos T regulatórios

mRNA: Ácido ribonucleico mensageiro

DNA: Ácido desoxirribonucleico

RNA: Ácido ribonucleico

cDNA: Ácido desoxirribonucleico complementar

TGF-B: Fator de Transformação do Crescimento Beta

TGF-BR1: Fator de Transformação do Crescimento Beta Receptor 1

TGF-BR2: Fator de Transformação do Crescimento Beta Receptor 2

IL: Interleucina

IFNG: Interferon Gama

TNF A: Fator de Necrose Tumoral Alfa

ARG1: Arginase 1

ARG2: Arginase 2

iNOS: Óxido nítrico sintase induzível

GLUT1: Transportador de Glicose do tipo 1

SUMÁRIO

1. LEVANTAMENTO BIBLIOGRÁFICO

A psoríase é uma doença inflamatória crônica, imunomediada, caracterizada por hiperplasia epidérmica, que acomete cerca de 1 a 3% da população mundial, afetando homens e mulheres, podendo surgir em qualquer época da vida, ocorrendo principalmente entre os 20 e 30 anos e entre os 50 e 60 anos de idade. Possui uma influência importante de fatores genéticos com uma participação bem evidenciada da hereditariedade no desenvolvimento da doença (CHRISTOPHERS, 2001; BÉRARD; NICOLAS, 2003).

A predominância do sistema imune adaptativo na patogênese da psoríase sempre foi evidenciada, porém a participação do sistema imune inato também tem ganhado destaque. Indicando, portanto, a existência de uma interação entre sistema imune inato e adaptativo, entre epiderme e tecido conjuntivo, envolvendo uma complexa rede de células e mediadores inflamatórios atuando sinergicamente na indução e manutenção do processo inflamatório (SCHÖN, BOEHNCKE, 2005).

A fisiopatologia da doença é caracterizada pela ativação imune inapropriada mediada por estímulos persistentes de linfócitos T, que liberam citocinas pró-inflamatórias causando manifestações cutâneas e articulares. Sabe-se que a doença tem o sistema imune inato e adaptativo atuando de forma exacerbada na produção destas citocinas pró-inflamatórias (RIVAS BEJARANO; VALDECANTOS, 2013). As quais na psoríase têm como alvo o queratinócito, levando a uma hiperproliferação destes e fazendo com que eles também produzam alguns fatores inflamatórios e precursores de inflamação (LOWES et al., 2014).

A doença caracteriza-se principalmente pelo aparecimento de placas eritemato-escamosas bem delimitadas, ocasionalmente pruriginosas. São lesões róseas ou avermelhadas, recobertas de escamas secas e esbranquiçadas que se alternam em períodos agudos com fases de piora e de melhora. As principais regiões afetadas são os joelhos, cotovelos, couro cabeludo, palmas das mãos e sola dos pés, podendo se estender pelo corpo (RYAN, 2008). A psoríase pode apresentar diferentes tipos clínicos, dentre elas, a variante clínica denominada psoríase vulgar ou psoríase em placa é a mais comum, afetando 85–90% dos pacientes (GRIFFITHS et al., 2007). Além da forma cutânea, a psoríase também pode acometer as articulações e está associada a várias outras comorbidades como doenças cardiovasculares, obesidade, hipertensão, diabetes, redução

na qualidade de vida, depressão entre outras (GARCIA-DIEZ et al. 2008; MACHADO-PINTO et al., 2016).

A pele humana, principal órgão acometido por lesões da psoríase, é considerado como tendo capacidade de órgão linfoide e possui uma variedade de tipos celulares e de mediadores do sistema imunológico que, em conjunto, promovem a proteção do corpo humano contra os agentes externos. Dentre essas células estão presentes células de Langherans, queratinócitos, células T, células fagocitárias, células endoteliais, as quais se comunicam através da secreção de diferentes citocinas, dentre as quais, a mais importante é o fator de necrose tumoral α. As possíveis patologias ocorrem quando há uma desregulação desses processos de proteção ao contra agentes externos. Ainda que a ativação do sistema imune seja um fenômeno de proteção que visa remover os antígenos prejudiciais, ela pode ocorrer de maneira constante, resultar num dano inflamatório crônico da pele e iniciar estados patológicos onde se inclui, por exemplo, a psoríase (PUIG et al., 2009; TRAUB, MARSHALL, 2007).

Contudo, a patogênese da psoríase ainda permanece incerta. Sabe-se que as células que auxiliam na diferenciação denominadas linfócitos T (LT), estão em evidência em todas as fases da doença, pois mantêm a resposta inflamatória ativa e o resultado às terapias que têm como alvo as células T, indicam que essas estão relacionadas à patogênese da doença (LIMA, 2006).

A totalidade da sua patogênese é difícil de ser elucidada, embora exista a teoria prevalente de que se trata de uma doença autoimune em que a epiderme e a proliferação capilar são afetadas pela liberação excessiva de citocinas produzidas por linfócitos resultantes de interações genéticas com fatores ambientais e o sistema imunitário. Fatores ambientais como uso de álcool, tabaco, infecções e *stress* podem originar uma resposta imunomediada exagerada na pele de indivíduos geneticamente susceptíveis, levando à proliferação epidérmica excessiva e alterações vasculares nas regiões cutâneas (KRUEGER et al., 2005).

A psoríase é uma doença inflamatória caracterizada pela expansão e ativação de células T helper (Th)1, Th17 e Th22, e pela produção de citocinas que lhes estão associadas, entre as quais se destacam o interferon (IFN- γ), o fator de necrose tumoral (TNF-α), a IL-17 e a IL-22 (LOWES et al., 2008, NOGRALES et al., 2008). Com uma intensa investigação no sistema imune conduziu na identificação de uma terceira linhagem de linfócitos T-helper, designados por Th17, devido à produção elevada de IL-17 (HARRINGTON et al., 2005) e pelas suas especificidades funcionais. A indução do

fenótipo Th17 a partir de células T-helper naive ocorre pela ação de múltiplas citocinas, incluindo TGF-β, IL-6, IL-21, IL-23, alguns fatores intracelulares como os fatores de transcrição STAT3 e RORγt, as próprias linhagens celulares linfocitárias Th1, Th2 e células T reguladoras (KIMURA et al., 2007; YANG et al., 2008). Os linfócitos Th17 desempenham um importante papel na eliminação de bactérias extracelulares e de fungos, interagindo com outras células imunitárias e outros tipos celulares. A produção de IL-17A, IL-17F e IL-22 pelos Th17 resulta no recrutamento e ativação de neutrófilos e macrófagos e na produção de fatores de ativação imunitária pelos queratinócitos, nomeadamente fator estimulador de colônias granulócito/macrófago, IL-8 ou TNF-α (KOGA et al., 2008).

As lesões psoriáticas são resultado da interação entre queratinócitos hiperproliferativos (KCs), células dendríticas inflamatórias (DCs), neutrófilos, mastócitos e células T, caracterizadas clinicamente por placas fortemente demarcadas, eritematosas e escamosas. Nas últimas três décadas, o modelo patogênico para a psoríase foi profundamente revisado de acordo com uma compreensão mais ampla e profunda dos mecanismos imunes que levam à formação da placa (CHIRICOZZI et al., 2018).

A imunopatogênese da psoríase pode ser dividida em duas fases: uma primeira em que ocorre a iniciação da resposta inflamatória, e uma segunda fase de manutenção dessa resposta. A fase de iniciação da doença acontece quando fatores ambientais específicos como infecções, traumas na pele, uso de algumas medicações levam ao estresse ou morte do queratinócitos. As lesões nos queratinócitos fazem com que ocorra a liberação de moléculas capazes de ativar células do sistema imune inato (células dendríticas mielóides e plasmocitóides, neutrófilos, monócitos, histiócitos e os próprios querátinócitos) (LANDE, 2007; NESTLE et al., 2005). Os queratinócitos presentes na epiderme possuem uma capacidade de recrutar e ativar as células T. Tanto a resposta imune inata quanto à adaptativa estão correlacionadas na cascata inflamatória do indivíduo com psoríase (ALLEN et al., 1999). Especificamente, as células T e as citocinas que produzem desempenham um papel central no desencadeamento e na perpetuação da resposta imunológica evidente na psoríase (CAPON et al., 2002).

A proliferação anormal dos queratinócitos juntamente com a diferenciação e mecanismos de defesa do hospedeiro são evidentes dentro das placas psoriáticas, em associação com a vascularização aumentada e o recrutamento pronunciado de leucócitos na derme subjacente. Acreditava-se que os queratinócitos eram os principais responsáveis celulares na patogênese da psoríase, isso porque a morfologia epidérmica (ou seja,

hiperplasia, acantose, hipogranulose, hiperqueratose, paraqueratose), estava alterada tão profundamente dentro das placas (GREB et al., 2016).

Esse tipo celular possui receptores para a maioria das citocinas sinalizadoras da psoríase, eles representam as células de tecido de resposta principal no microambiente psoriásico. Respondem às citocinas psoriáticas pela proliferação e amplificação da inflamação através da produção de outras citocinas, quimiocinas, fatores estimulantes da proliferação e outros produtos pró-inflamatórios (CHIRICOZZI et al., 2011, NOGRALES et al., 2008, HARPER et al., 2009). Cada citocina modula distintas vias de resposta de queratinócitos com certo grau de sobreposição na indução da expressão gênica (CHIRICOZZI et al., 2011, NOGRALES et al., 2008, HARPER et al., 2009, GUILLOTEAU et al., 2010).

Certas citocinas, como a IL-17 e o TNF-α induzem fortemente a síntese de mediadores pró-inflamatórios com efeitos aditivos e sinérgicos na expressão do gene dos queratinócitos (CHIRICOZZI et al., 2011, PRIGNANO et al., 2015), apresentando um efeito semelhantemente a IL-22 e outros membros da família de citocinas IL-20 (isto é, IL-19 e IL-20) estimulam a hiperplasia de queratinócitos, promovendo a espessura da epiderme (WOLK et al., 2006, WOLK et al., 2009, DONETTI et al., 2016).

Efeitos de hiperproliferação das células basais da epiderme levam a uma resposta exagerada do sistema imune inato da epiderme. Nos doentes com psoríase, devido a um ou mais fatores estimuladores, ocorre ativação das células dendríticas apresentadoras de antígeno (APC) - responsáveis pelo processamento de antígenos e sua apresentação às células T. As células dendríticas (células de Langerhans) secretam IL-12, cuja ligação a receptores de superfície das células T permite a sua diferenciação em células efetoras, e TNF-α, potente citocina pró-inflamatória (LOWES et al., 2014; MARQUES et al., 2010; SANCHEZ, 2010).

Semelhante a outras doenças auto-imunes, a genética da psoríase é complexa e multifatorial. Há evidências claras de um componente genético importante para a psoríase. Isto é apoiado por estudos de gêmeos e familiares (WUEPPER et al., 1990). A taxa de concordância de gêmeos monozigóticos é de aproximadamente 70% e para gêmeos dizigóticos é de cerca de 20% (BOWCOCK, 2005).

Muitos estudos genéticos demonstram o principal papel do sistema imunológico na patogênese da psoríase. Estudos de associação ampla do genoma (GWAS) identificaram aproximadamente 50 locais genéticos associados ao risco de psoríase (TSOI et al., 2012; ANBUNATHAN, BOWCOCK, 2017).

As áreas de cromossomos que se acreditava abrigarem genes da psoríase eram inicialmente denominadas *locos de PSORS* (**psor**iasis- susceptibility). Existem pelo menos 12 locais diferentes de *PSORS* que foram identificados principalmente através da análise de ligação de famílias de psoríase afetadas (LOWES, et al; 2014). Porém o gene ou genes para a maioria dos *PSORS* que são responsáveis pela susceptibilidade não é conhecido.

O primeiro gene que se descobriu ser significativamente associado à susceptibilidade à psoríase foi o HLA-Cw6, que está localizado no *PSORS1* na posição cromossômica 6p21.3 (BOWCOCK, 2005; ELDER, 2006). O HLA-Cw6 é encontrado em cerca de 4-16% dos controles saudáveis (GOURRAUD et al., 2014), e em cerca de 20% a mais de 50% dos casos de psoríase, dependendo da população em estudo. O HLA-Cw6 codifica um alelo do complexo principal de histocompatibilidade I (MHCI). As moléculas de MHCI estão presentes em quase todas as células nucleadas e são moléculas-chave para a vigilância imunológica, uma vez que apresentam peptídeos intracelulares (tanto peptídeos próprios quanto não próprios) para o sistema imunológico. O MHCI também é crítico para o estímulo das células T CD8 + e subsequente direcionamento citolítico das células. Esta descoberta apoiou o importante papel das células T na patogênese da psoríase (BOWCOCK, 2005; ELDER, 2006).

Numerosos estudos demonstraram que existe uma forte ligação entre certos polimorfismos do fator de necrose tumoral-α (TNF-α) ou interleucina-23 (IL-23) e a gravidade da doença ou a resposta ao tratamento com anti-TNF-α. Outro grupo de interleucinas com implicações comprovadas na patogênese da psoríase é a família da IL-17 (BATALLA et al, 2015; LONNBERG et al., 2014; MALAKOUTI et al., 2014).

O Fator de Necrose Tumoral Alfa (TNF-α), é uma citocina pró-inflamatória produzida por diferentes tipos celulares, como os linfócitos T ativados, queratinócitos e células de Langerhans (WOLLINA et al., 2008). Estudos apontam esta citocina como precursora dos processos que envolvem inflamação, imunidade, crescimento, diferenciação celular e apoptose (ETTEHADI et al., 1994). Além disso, desempenha importantes atividades como a amplificação da produção de outras citocinas pró-inflamatórias, indução da maturação de células de Langerhans, aumento da proliferação dos queratinócitos e promoção de moléculas de adesão endotelial e fator de crescimento endotelial vascular (WINTERFIELD et al., 2004). O papel exato do TNF-α ainda não está claro na patogenia da psoríase, mas *a* terapia anti-TNF-α é altamente eficaz na psoríase, indicando que essa citocina tem, juntamente com o IFN-γ um papel central na

patogênese da doença (BONIFATI et al., 1999). Em conjunto com o IFN-γ o TNF-α induz rapidamente a expressão das citocinas IL-6, IL-8, IL-12 e IL-23 a criarem uma ligação importante na rede de citocinas na patogênese da psoríase (GUDJONSSON et al., 2004). Lesões psoriáticas demonstram níveis elevados de TNF-α em todas as camadas da epiderme (KRISTENSEN et al., 1993; ETTEHADI et al., 1994). Níveis reduzidos de TNF-α foram detectados na pele lesional e no soro de pacientes com psoríase, em comparação com a pele não lesional ou saudável, de pacientes com terapia anti- TNF-α (CALDAROLA et al., 2009; ARICAN et al., 2005).

Esta citocina aparece como a primeira citocina a ser destinada com sucesso por anticorpos monoclonais terapêuticos ou proteínas de fusão para o tratamento da psoríase (CHIRICOZZI et al., 2018). Com isso destaca-se que o TNF-α pode induzir um loop de feedback positivo através da indução do mRNA do TNF-α nos queratinócitos, que se pensa resultar na propagação da doença por efeitos em imunócitos e nos queratinócitos (TAN et al., 2007).

Outra citocina pró-inflamatória com papel extremamente importante na psoríase é a interleucina IL-17, que é produzida pelo subconjunto T helper 17 (Th17) de células T $CD4^+$. Pertence à família de citocinas pró-inflamatórias que incluem 6 membros IL17A, B, C, D, E e F, com ativos homodímeros ou heterodímeros (GAFFEN et al., 2006). A IL-17A é considerada a citocina mais relevante desta classe, pois mostra a maior atividade biológica e efeitos inflamatórios marcados (GAFFEN et al., 2009). Supõe-se que a IL-17C, um membro mais novo da família IL-17, também esteja envolvida na patogênese da psoríase. Biópsias de pele de lesões psoriáticas revelaram expressão significativamente aumentada de IL-17C, até 125 vezes maior que a de IL-17A (FUJISHIMA et al., 2010).

A IL-17 é a principal citocina efetora das células Th17, que estimula os queratinócitos a produzir quimiocinas, citocinas, e outros mediadores pró-inflamatórios, permitindo assim, que a IL-17A faça a ponte entre o sistema imunológico inato e adaptativo, para sustentar a inflamação crônica (AGGARWAL, GURNEY, 2002). Tendo os queratinócitos como principal alvo, esta interleucina se liga aos receptores de IL-17 presentes nos queratinócitos e os ativam, liberando várias quimiocinas que agem no perpetuamento do processo inflamatório (MAHIL et al., 2016. Esta citocina é secretada pelas células Th17, mas também por outras células do sistema inato e adaptativo, como neutrófilos, mastócitos, linfócitos $CD8^+$, linfócitos $ROR\gamma t^+$ e células linfoides inatas Tγδ, são as principais produtoras de IL-17 (PANTELYUSHIN et al., 2012). A IL-17A

estimula os fibroblastos, células epiteliais e endoteliais a produzir mediadores inflamatórios (BATALLA et al., 2015, STARNES et al., 2001, ARISAWA et al., 2008). Da mesma forma, ao criar uma conexão entre o sistema imune inato e adaptativo, mobiliza, recruta e ativa neutrófilos (KAWAGUCHI et al., 2004).

Na psoríase foi detectado um aumento de IL-17 na pele dos doentes, assim como de IL-22, citocina também produzida pelas células Th17 que, aparentemente, contribui para a indução da proliferação de queratinócitos e para a produção de peptídeos antimicrobianos e citocinas (ZHENG et al, 2007). A IL-17 desencadeia alterações nos queratinócitos, características da psoríase através da sinergia desta citocina com outros mediadores como o IFN-γ, o TNF-α ou a IL-22 (TEUNISSEN et al., 1998, CHIRICOZZI et al., 2013). Estudos com biópsia cutânea mostram que as lesões da psoríase contêm células que secretam IL-17, especialmente linfócitos Th17, em maior número comparado à pele normal (BATALLA et al., 2016, ROOSTAEYAN et al., 2017, WILSON et al., 2007, ASARCH et al., 2008). Com isso, a IL-17A é altamente expressa na pele afetada em comparação com o tegumento não afetado (PRIETO-PEREZ et al., 2015). Pacientes com psoríase têm níveis plasmáticos aumentados de IL-17, bem como níveis mais altos de células produtoras de IL-17 circulantes, em comparação com pessoas saudáveis (HARPER et al., 2009, WILSON et al., 2007, KAGAMI et al., 2009, BIALECKA et al., 2016).

Mesmo com evidências crescentes de envolvimento de células Th17 na patogênese da psoríase, os efeitos relativos das citocinas Th17 - IL-17 e IL-22 e da citocina Th1 - IFN-γ na pele, são desconhecidos (NOGRALES et al., 2008). Lesões de pele psoriáticas são relatadas como tendo expressão gênica aumentada de IL-23, IL-17 e IL-22, conduzindo assim, os investigadores a pesquisar mais profundamente o potencial envolvimento de células Th17 na psoríase (LEE et al., 2004, LOWES et al., 2008, LI et al., 2007).

Da mesma família de pró-inflamatórias, podemos destacar a interleucina 23 (IL-23), é um heterodímero composta por duas subunidades, p19 e p40. Pertence à família de citocinas IL-6/IL-12 (CHO et al., 2006). A IL-23 tem sido associada à patogênese da inflamação autoimune (WIEKOWSKI et al., 2001). Diferentes tipos de células, incluindo queratinócitos e células apresentadoras de antígenos, como células dendríticas mielóides dérmicas, macrófagos e células de Langerhans epidérmicas são capazes de produzir IL-23 (MCGEACHY et al., 2007).

Atualmente, acredita-se que a psoríase seja uma doença autoimune prototípica da pele com um papel central no eixo interleucina 23/T-helper 17 (IL-23/Th17) (LYNDE et al., 2014; MARTIN et al., 2013). A centralidade da IL-23 está intimamente ligada à IL-17, que representa a principal citocina efetora na sua via de sinalização (LANGRISH et al., 2005, DI CESARE et al., 2009).

Notavelmente, estudos de associação genômica ampla reconheceram IL-23p19 e IL-23R como genes de susceptibilidade (LOWES et al., 2013, CAPON et al.,2007). A sinalização de IL-23 promove respostas imunes celulares promovendo a sobrevivência e a expansão de um subconjunto de células T recentemente identificado que expressa a IL-17, que protege os epitélios contra patógenos microbianos (BETELLI et al., 2007). Enquanto a IL-12 induz o desenvolvimento de células Th1, que produzem interferon-γ, a IL-23 está envolvida na diferenciação de células Th17, em um contexto pró-inflamatório e especialmente na presença de TGF-β e IL-6. As células Th17 ativadas produzem IL-17A, IL-17F, IL-6, IL-22, TNF-α. Macrófagos inflamatórios expressam IL-23R e são ativados pela IL-23 para produzir IL-1, TNF-α e a própria IL-23 (DUVALLET et al., 2011).

Uma sinalização desregulada de IL-23 poderia levar a respostas imunológicas crônicas e inapropriadas que atingem as células epiteliais, talvez ajudando a explicar a inflamação relativamente específica da pele observada na psoríase (DUERR et al., 2006). Além disso, na pele com lesão psoriática, ocorre uma expressão aumentada de IL-12p40 e IL-23p19 em comparação com a pele não lesionada (PISKIN et al., 2006, LEE et al., 2004). A expressão aumentada de IL-23 na pele psoriática está associada a uma infiltração acentuada de células dendríticas mielóides (células dendríticas CD11c+), que são as principais fontes de IL-23 (ZABA et al., 2009). Consistentemente, os níveis séricos de IL-23 foram encontrados significativamente maiores em pacientes com psoríase do que em controles saudáveis (FOTIADOU et al., 2015). O papel da IL-23 e do IL-23R na inflamação cutânea tem sido investigado tanto em camundongos quanto em humanos. A aplicação de uma injeção intradérmica de IL-23 em camundongos levou a eritema, endurecimento e proeminentes vasos sanguíneos papilares dérmicos com características histopatológicas parecidas com a psoríase (CHAN et al., 2006). As características morfológicas das lesões cutâneas induzidas por IL-23 foram mais severas do que as induzidas por IL-12 (ZHENG et al., 2007). Esses dados indicam que a produção de IL-23 ocorre em locais inflamatórios da pele e é mediada por células imunes residentes no tecido e/ou recrutadas, tais como DCs e, possivelmente, KCs (PISKIN et al., 2006). Outra

evidência que apoia um papel patogênico da IL-23 na psoríase vem dos dados clínicos. Em um estudo os autores mostraram que os agentes anti-TNF-α são capazes de modular os níveis de mRNA de IL-23p19 e IL-12p40 e o infiltrado inflamatório na pele psoriática (ZABA et al., 2007).

Uma citocina da família da IL-10 que possui função pró-inflamatória no fígado, pâncreas, intestino e pele, e tem participação na psoríase, é a IL-22 (WOLK et al., 2004). Expressa principalmente por células T ativadas, mastócitos e células natural killer (NK) e atua por meio de um receptor heterodimérico (DUMOUTIER et al., 2001; WOLK et al., 2002). Produzida por várias células da linhagem linfoide, pertencentes ao sistema imune adaptativo e inato: células T αβ, células T γδ, células T natural killer e células linfoides inatas (DUDAKOV et al., 2015; JIA, WU, 2014).

Estudos demonstram que esta citocina assume importantes funções de cross-talk entre células imunes e epiteliais (SABAT et al., 2014; DUDAKOV et al., 2015). Além de demonstrar a participação para induzir peptídeos antimicrobianos e quimiocinas pró-inflamatórias, esta citocina também inibe a diferenciação terminal dos queratinócitos, assim a IL-22 tem demonstrado contribuir amplamente para a inflamação e remodelação epitelial da pele psoriática (BONIFACE et al., 2005).

Esta citocina é regulada pela proteína de ligação IL-22 (IL-22BP), que é um receptor de cadeia única solúvel codificada pelo gene IL22RA2 (DUMOUTIER et al., 2001). Mesmo sendo expressa em células endoteliais, fibroblastos dérmicos, os queratinócitos são o principal alvo para a IL-22 na pele (WOLK et al., 2006). Sendo assim, observa-se que a expressão do receptor IL-22 está aumentada na epiderme da pele lesionada psoriática em comparação com a pele normal (BONIFACE et al., 2007). Estudos apontam que o aporte patogênico desta citocina está ligado a suas interações positivas com outros sinais de citocinas (WAWRZYCKI et al., 2019).

Como vem sendo demonstrado, as citocinas pró-inflamatórias tem uma participação importante na psoríase, e dentre essas, também devemos destacar o interferon gama (IFN-γ), proveniente de linfócitos Th1, que desempenham um papel muito importante na imunidade inata e adaptativa contra infecções virais e intracelulares (SAUNDERS, JETTEN, 1994). Estudos destacam que esta citocina no ambiente psoriático estimula a liberação das citocinas IL-1 e IL-23, que em seguida desencadeia a ativação e diferenciação de linfócitos Th17 (AGGARWAL et al., 2003; PARK et al., 2005). Estudos apontam que esta citocina apresenta um aumento significativo no soro de pacientes psoriáticos quando comparados aos controles saudáveis (SZEGEDI et al., 2003;

GOMI et al., 1991). Também é considerada como uma citocina chave na patogenia da psoríase (SZEGEDI et al., 2003), pois o IFN-γ estimula a liberação de várias citocinas pró-inflamatórias, como IL-1, IL-6, IL-8, TNF-alfa e mediadores inflamatórios, além de induzir a expressão de ICAM-1 e HLA-DR, vascular em queratinócitos e células endoteliais (KURTOVIC et al., 2018). Estudos em tecidos psoriáticos lesionados *ex vivo*, demonstram que o IFN-γ controla a expressão de aproximadamente 400 genes, através da ativação do transdutor de sinal e ativador da transcrição 1 (STAT1), um fator de transcrição com domínio do IFN-γ (CHIRICOZZI et al., 2014; CHIRICOZZI et al., 2017). Já em estudos *in vitro*, a estimulação com IFN-γ modifica a expressão de aproximadamente 1200 genes em queratinócitos de monocamada (NOGRALES et al., 2008). Os mecanismos sugeridos são que o IFN-γ medeia interações entre células T inflamatórias e queratinócitos, facilitando a migração de células T para a epiderme lesional (LIU et al., 2007). O nível de IFN-γ avaliado pelo método de ELISA em epiderme psoriática demonstra o aumento quando comparados às concentrações na epiderme saudável (OVIGNE et al., 2001).

No ambiente psoriático também ocorre a presença de citocinas imunorreguladoras e dentre elas se destaca, a interleucina 10 (IL-10), que foi descoberta em 1989. É uma citocina imunorreguladora pleiotrópica, e possui propriedades anti-inflamatórias. Produzida tanto por células da imunidade inata como da adquirida, a IL-10 é secretada principalmente por macrófagos, mas também por linfócitos T helper 1 (Th1) e Th2, Treg, células dendríticas, células T citotóxicas, linfócitos B, monócitos e mastócitos (GASTL et al., 1993; PISA et al., 1992).

A atividade da IL-10 é mediada pelo receptor de IL-10 (IL-10R) que é um membro da família de receptores de citocinas do complexo principal de histocompatibilidade (MHC) de classe II (TRIFUNOVIC et al., 2015). A IL-10 inibe a produção e expressão de importantes citocinas pró-inflamatórias, moléculas de adesão co-estimulatórias e quimiocinas, como IL-1, IL-6, IL-8, IL-12 e fator de necrose tumoral alfa (TNF-α) (ASADULLAH et al., 1998). Exerce um papel regulador, no qual inibe os danos ao hospedeiro em função da resposta imune ao patógeno, mas opostamente pode aumentar certas funções da resposta imune (O´GARRA et al., 2008). Baixos níveis de IL-10 foram observados em diversos estudos (ASADULLAH et al., 2004; KARAM et al., 2014) e acredita-se que desempenhe um papel fundamental na patologia e no curso clínico da psoríase (TRIFUNOVIC et al., 2015). Na psoríase, observa-se uma deficiência relativa

de IL-10 no soro e na pele, portanto, parece ser um fator importante na patogênese psoriática (ASADULLAH et al., 2004).

Outra citocina que regula ou tenta regular o ambiente inflamatório na psoríase é o Fator de Crescimento Transformador Beta, que pertence a uma família de fatores de crescimento envolvidos em várias funções celulares essenciais. As proteínas do fator de crescimento transformador β (TGF-β) representam uma importante família de citocinas que desempenham um papel forte na regulação de funções celulares, como proliferação celular, diferenciação, apoptose, adesão, invasão e resposta imune (DE CAESTECKER et al., 2000; MASSAGUE, 2008). A proteína apresenta três isoformas: TGFβ1, TGFβ2, TGFβ3 que são expressas em mamíferos codificadas por genes distintos (ROBERTS et al., 1992). Após sua ativação o TGF-β desempenha várias funções biológicas através da ligação a receptores específicos de transmembrana, dos quais apresentam forte ligação. O TGFβ do tipo I (TGF-BR1) e tipo II (TGF-BR2) contêm serina-treonina quinases em seus domínios intracelulares (ELLIOTT et al., 2005). Também participa de vários processos durante a cicatrização das lesões como proliferação de fibroblastos, inflamação, angiogênese, síntese de colágeno e remodelação da nova matriz extracelular (HE et al., 2006, HE et al., 2004).

Existe a participação de diversas enzimas no ambiente psoriático e na tentativa de combate a lesões psoriáticas, entre elas, as óxido nítrico sintase (iNOS), que são hemeproteínas citoplasmática que catalisam a conversão de L-arginina em L-citrulina e óxido nítrico (NO) (XIE et al., 1992). É a mais importante proteína da família do óxido nítrico sintase, a qual é capaz de produzir grandes quantidades de óxido nítrico (NO). Além de suas funções como potente vasodilatador e neurotransmissor, o NO é importante na inflamação e imunidade (SALVEMINI et al., 1998). É expressa em várias células do sistema imune como, por exemplo, em macrófagos, células endoteliais e por citocinas como IL-1β, TFN-α, IFN-γ, IL-6 (FÖRSTERMANN et al., 1994). Demonstram que o óxido nítrico é a molécula de sinalização para o crescimento de queratinócitos e desempenha um papel fundamental na psoríase (BRUCH-GERHAZ et al., 1998). Assim, o NO age na eliminação de patógenos, e participa do processo de cicatrização tecidual. Também participa do processo de estimulação gênica, proliferação e diferenciação celular, especialmente nos queratinócitos, promovendo assim a re-epitelização celular. E durante o processo de cicatrização, a produção de NO é seguida pela indução da produção de colágeno (WITTE et al., 2002). Estudos apontam o NO como um mediador lábil e pode ser detectado em níveis elevados em placas de psoríase, na presença de algumas

citocinas como IL-8, IL-6, IL-17, IFN-γ e TNF-α que resulta em aumento na transcrição de iNOS e arginase através da sinalização de NFκ B (ABEYAKIRTHI et al., 2010). Durante sua ação o NO executa várias funções nas células, por exemplo: nos macrófagos aumenta sua atividade fagocitária contra bactérias e células neoplásicas (LIEW et al., 1991). Já nas células epiteliais, atua como um modulador endógeno da adesão e infiltração linfocitária (KRONCKE et al., 1997).

Ainda participando no metabolismo do óxido nítrico, participam a arginase 1 (ARG-1) e arginase 2 (ARG-2), que são metaloenzimas binucleares de manganês que catalisam a hidrólise de L-arginina em L-ornitina e ureia, ambas as isoenzimas são codificadas por dois genes separados (DIZIKES et al., 1986). Em seres humanos, o gene ARG-1 mapeia o cromossomo 6q23 e codifica uma proteína de 322 aminoácidos (DIZIKES et al., 1986; SPARKES et al., 1986), enquanto o gene ARG-2 mapeia o cromossomo 14q24.1 e codifica uma proteína de 354 aminoácidos (GOTOH et al., 1996, GOTOH et al., 1997). No nível subcelular, ARG-1 está localizado principalmente no citoplasma e ARG-2 na mitocôndria (JENKINSON et al., 1996). Assim, essas isozimas diferenciam entre si em relação à distribuição dos tecidos, localização subcelular e reatividade imunológica (JENKINSON et al., 1996). Estas duas isoenzimas compartilham aproximadamente 60% de homologia de sequência de aminoácidos, que são críticas para a função metabolizadora da L-arginina (DIZIKES et al., 1986).

A ARG-1 é uma enzima citosólica, expressa especialmente no fígado, e induzível por citocinas em muitos tipos celulares (EFRON, BARBUL, 1998), que tem como principal função a remoção do nitrogênio excessivo produzido pelo metabolismo através do ciclo hepático da ureia, que é tóxico ao nosso organismo (CROMBEZ, CEDERBAUM, 2005). Já a ARG-2 é uma proteína mitocondrial que possui uma ampla distribuição tecidual, com maior expressão nos rins, intestino delgado e cérebro (MORI, GOTOH, 2000). Porém nestes órgãos as funções desta arginina não são claras. A melhor caracterização da função ARG-2 é feita em células endoteliais vasculares nas quais a isoenzima, semelhante à ARG-1, metaboliza L-arginina em ureia e L-ornitina, o que limita a biodisponibilidade de L-arginina para geração do NO vasoprotetor via eNOS, resultando em disfunção endotelial vascular (XIA et al., 1996; KIM et al., 2009).

Em um estudo, os pesquisadores avaliaram o possível efeito inibitório da atividade da ARG-1 na síntese de NO derivado de iNOS em culturas de queratinócitos humanos, o que demonstrou que a atividade da ARG-1 pode realmente restringir a disponibilidade de substrato intracelular para iNOS e, assim, dificultar significativamente a taxa de produção

de NO nos queratinócitos epidérmicos, indicando que a superexpressão de ARG-1 pode ser um mecanismo molecular para a hiperproliferação de queratinócitos na psoríase por limitação da atividade da iNOS (BRUCH-GERHARZ et al., 2003).

Tudo o que ocorre nesse ambiente necessita da participação de glicose e atuando na sua regulação e participando da regulação desses processos, existe o transportador de glicose (GLUT-1), que pertence à superfamília maior facilitadora (MFS) de transportadores de membrana. São proteínas de membrana que mediam a transferência de açucares através das membranas celulares (JOOST et al., 2002). GLUT-1 é uma proteína de 492 resíduos de aminoácidos e contém um único oligossacarídeo N-ligado (MUECKLER et al., 1985).

A glicose é a principal fonte de energia para as células, e o transportador de glicose 1 é o transportador de glicose mais comum em humanos (GLUT-1). GLUT-1 facilita o transporte de glicose através das membranas plasmáticas das células de mamíferos. Os níveis de expressão de GLUT-1 nas membranas celulares são aumentados por níveis reduzidos de glicose e diminuídos por níveis aumentados de glicose (GATENBY et al., 2007). O GLUT-1 é o transportador de glicose facilitador mais amplamente expresso e regula a captação basal de glicose na maioria dos tecidos, incluindo as células basais da epiderme (CURA, CARRUTHERS, 2012; GHERZI et al., 1992). Estudos anteriores indicaram que a regulação positiva do GLUT-1 contribuiu na melhoria do metabolismo da glicose, necessária para a rápida proliferação nas células cancerígenas (WEINER et al., 2004). Embora as consequências metabólicas do aumento do transporte de glicose não sejam compreendidas, a expressão de GLUT-1 aparentemente tem uma função clínica significativa em vários tumores. Tanto a psoríase quanto os tumores benignos ou malignos têm características comuns, como proliferação celular excessiva, angiogênese e presença de regiões de hipóxia (DIERCKX, VAN DE WIELE, 2008).

Com o papel de promover a proliferação de queratinócitos, a expressão de GLUT-1 é aumentada na cicatrização de feridas, em placas psoriáticas ou após hiperplasia induzida por luz ultravioleta (UV) (TOCHIO et al., 2013). Estudos relatam que o GLUT-1 é altamente expresso na epiderme de lesões psoriáticas envolvidas e durante a cicatrização, onde ocorre proliferação celular excessiva (TAO et al., 2008; ELSON et al., 2000). Em um estudo imunohistoquímico com biópsias de pacientes com psoríase, demonstrou um aumento significativo na expressão imunohistoquímica do GLUT-1 e na expressão do mRNA do GLUT-1 na pele lesional de pacientes com psoríase, em comparação com a pele não-lesional e a pele normal dos indivíduos controle. Sugerindo,

que a regulação positiva do GLUT-1 nas lesões de psoríase poderia participar da patogênese da psoríase através da facilitação do transporte de glicose (HODEIB et al., 2018).

Apesar de não haver atualmente uma cura definitiva para a psoríase, existe um conjunto de terapêuticas eficazes no controle da doença, dependendo este da adesão à terapêutica por parte dos doentes. Em casos moderados e graves da doença, o tratamento sistêmico é bastante utilizado, ele consiste em uso de medicamentos imunosupressores, que inibem a resposta imune, diminui a inflamação e o infiltrado celular, induzindo a uma melhora significativa das lesões psoriáticas (ZABA et al., 2007).

O metotrexato (MTX) tem sido utilizado no tratamento da psoríase moderada a grave (HERMAN et al., 2005). O metotrexato é um antimetabólito, análogo do ácido fólico, cujos efeitos podem ser classificados em efeitos antiproliferativos e anti-inflamatórios (SRAMEK et al., 2017). No centro da via anti-inflamatória está um nucleotídeo de purina conhecido como adenosina, que tem capacidade para combater o processo inflamatório (CHAN, CRONSTEIN, 2002). Os efeitos antiproliferativos, antineoplásicos e citotóxicos são baseados na diminuição da formação de ácidos nucleicos nas células T ativadas e nos queratinócitos (PATHIRANA et al., 2009). Na psoríase o MTX também aparece como um agente imunomodulador. Os efeitos imunomoduladores do MTX podem ser explicados pela diminuição da inflamação mediada por células T em várias etapas (SALEH et al., 2010). Assim, o MTX com sua função imunomodulatória reduz a produção de IL-1 e diminui a densidade de células de Langherhans na epiderme (BOURNERIAS, CHOSIDOW, 1994, WEINSTEIN et al., 1990). O MTX inibe o crescimento de queratinócitos e é capaz de regular negativamente a expressão endotelial das moléculas de adesão celular ICAM-1 e E-selectina (DAHLMAN-GHOZLAN et al., 2004). Estudos *in vitro* demonstraram que o MTX diminui os marcadores associados à proliferação nas biópsias de pele de pacientes com psoríase (YAZICI et al., 2005).

2. HIPÓTESE

Moléculas moduladoras da inflamação são diferencialmente expressas em biópsias de tecido lesional e não lesional de pacientes com psoríase.

3 OBJETIVOS

OBJETIVO GERAL

Analisar a expressão gênica *in situ* de mRNA de moléculas moduladoras da resposta imune e da inflamação na psoríase em placa.

OBJETIVOS ESPECIFICOS

- Avaliar a expressão gênica *in situ* pelo mRNA da IL-17, 1L-23, IL-22, IL-10, IFN-γ, TNF-α, TGF-βR2, ARG-1, ARG-2, iNOS e GLUT-1 em biópsias de tecido lesional e não lesional de pacientes com psoríase, comparando com tecido saudável.

- Avaliar a expressão gênica *in situ* de mRNA de IL-17, 1L-23, IL-22, IL-10, IFN-γ, TNF-α, TGF-βR2, ARG-1, ARG-2, iNOS e GLUT-1 nos pacientes em uso de metotrexato e sem uso de medicação.

4 MATERIAIS E MÉTODOS

Este trabalho foi aprovado pelo Comitê de ética e Pesquisa (CEP) da Universidade Uberaba (UNIUBE), sob protocolo de número 63049316.1.100005145 e foi realizado na Universidade de Uberaba e no Hospital das Clínicas da Universidade Federal do Triângulo Mineiro (UFTM), Uberaba, Minas Gerais, Brasil. (ANEXO 01)

Todos os pacientes elegíveis foram informados da natureza do estudo, os potenciais riscos e benefícios de sua participação no estudo e assinaram o Termo de Consentimento Livre e Esclarecido (TCLE). (ANEXO 02)

4.1. Casuística

Os pacientes que necessitaram de confirmação do diagnóstico foram removidos um fragmento da pele utilizando um *punch* de 5mm, coletado na lesão, que foi fixado em formaldeído 10% para diagnóstico anatomopatológico.

A casuística foi constituída por 26 pacientes. Destes, 21 pacientes diagnosticados com psoríase moderada e grave (PASI> 10). Destes, foram realizadas coletas de biópsias de tecido lesional e tecido não lesional do mesmo paciente, 15 estavam em uso de metotrexato (MTX) e 6 estavam sem uso de medicação, e como grupo controle foram selecionados 5 pacientes saudáveis, que passaram por um procedimento cirúrgico de reparação estética, cuja incisão foi realizada em região da mama, submetida ao tratamento cirúrgico. Estas biópsias foram colocadas em solução de *RNA-latter* e congeladas em -90º para realização do q-PCR.

4.2. Critérios de inclusão pacientes com psoríase em placas:

- Apresentaram diagnóstico clínico de psoríase em placas, associado ou não a outras comorbidades como artrite psoriásica, por exemplo;
- Apresentaram psoríase em placas de grau moderado a grave, calculado pelo "Psoriasis Area Severity Index – PASI";
- Apresentaram valor de *score* medido pelo PASI maior que 10 para ser considerada uma psoríase moderada a grave;
- Não estar em uso de nenhuma medicação imunobiológica, caso utilizado previamente, deve estar com período mínimo de 6 meses sem uso das mesmas.

- Estavam fazendo uso de qualquer outra medicação sistêmica não imunobiológica para psoríase, como metotrexato, por exemplo;
- Aos pacientes em uso de metotrexato, estavam fazendo uso da medicação há pelo menos 3 meses de tratamento;
- Deve ter lido e concordado em assinar o TCLE.

4.3. Critérios de exclusão pacientes com psoríase em placas:

- Pacientes com psoríase em placas, mas com PASI<10;
- Pacientes com outros de tipo de psoríase que não de placas;
- Pacientes que estavam fazendo uso de imunobiológicos;
- Pacientes que não queiram assinar o Termo de Consentimento.

4.4. Metodologia da Expressão Relativa dos Genes IL-17, IL-23, IL-22, IL-10, IFN-γ, TNF-α, TGF-βR2, ARG-1, ARG-2, iNOS e GLUT-1

Extração de RNA

A extração do RNA total foi realizada utilizando-se kit de extração de RNA (RNA SV Total RNA Isolation System, Promega - EUA), de acordo com as recomendações do fabricante. Os fragmentos de tecidos, armazenados em nitrogênio, foram processados por maceração mecânica. Os fragmentos resultantes deste procedimento foram transferidos para tubo eppendorf contendo tampão de lise, e misturados por inversão; foi então adicionado 350µL de tampão de diluição. As amostras foram aquecidas em banho-maria a 70ºC por 3 minutos e centrifugadas a 10 minutos a 13000xg à 4ºC. Após esta centrifugação o sobrenadante foi transferido para novos tubos eppendorfs e adicionado 200µL de etanol a 95%. O material foi transferido para um conjunto de separação do kit, contendo uma membrana de separação, sendo posteriormente centrifugado por 1 minuto a 13000xg à 4ºC; o RNA foi lavado, por 1 minuto, por centrifugação (13000xg à 4ºC) em 600µL de tampão apropriado. A presença de DNA contaminante foi eliminada com tratamento com DNase por 15 minutos à temperatura ambiente. Após esta incubação foi adicionado 200µl de DNAse *stop* e centrifugado por 1 minuto a 13000xg à 4ºC. O material foi novamente lavado por 2 vezes (1 minuto a 13000xg à 4ºC). Esta membrana separadora contendo o RNA foi transferida para outro tubo e adicionado 30µL de água livre de nucleasse, e centrifugado por 1 minuto a 13000xg à 4ºC, para quantificação e confecção de DNA complementar (cDNA).

Confecção de cDNA

O cDNA foi confeccionado a partir de 1 µg de RNA, 0,5 µg de Oligo dT (Promega - EUA) e água ultra pura (Milli-Q) autoclavada. Esse material foi levado ao termociclador PTC-100 (MJ Research, Inc - EUA) para um ciclo de 5 minutos a 70ºC. Após resfriamento imediato, foram adicionados dNTP (2,5mM), transcriptase reversa M-MLV RT (Improm II, Promega - EUA) e tampão para reação M-MLV-5x Buffer (Promega - EUA). Essa reação foi levada ao termociclador para mais um ciclo de 1 hora à 42ºC, seguido de 3 minutos a 10ºC. No final, foram adicionados ao cDNA confeccionado 75µL de água ultra pura autoclavada, sendo estas amostras congeladas em seguida a -20ºC, até o momento de uso.

Reações de PCR Quantitativo (qPCR)

A expressão quantitativa de mRNA dos genes *IL-17* (cod.Hs001743383), *IL-23* (cod.Hs00900828), *IL-22* (cod.Hs01574154), *IL-10* (cod.Hs00961622), *IFN-γ* (cod.Hs00989291), *TNF-α* (cod.Hs00174128), *TGF-βR2* (cod.Hs00234253), *ARG-1* (cod.Hs00163660), *ARG-2* (cod.Hs00982833), *iNOS* (cod.Hs01075529) e *GLUT-1* (cod.Hs00892681), foram analisadas por reações de PCR em tempo real, nas amostras de cDNA de fragmento dos pacientes com diagnóstico de psoríase e tecido controle saudável. Foram utilizados *primers* e sondas do sistema *Taqman* (*Applied Biosystems* – EUA) no aparelho de PCR em tempo real *StepOnePlus* (*Applied Biosystems* – EUA) com *primers* adequados para tais reações e utilizando a β-actina (cod.Hs01060665) como controle. O cDNA sintetizado a partir do RNA mensageiro foi utilizado de acordo com as instruções do fabricante. Os resultados foram analisados com base no valor de CT (*cycle threshold* – ou ciclo limiar) e a fórmula aritmética para alcançar a quantificação relativa foi $\Delta\Delta Ct = \Delta$ Ct (*treated*) – Ct (*control*) (LIVAK, SCHMITTGEN 2001).

4.5 Análise estatística

Os dados foram analisados utilizando o Software *GraphPad Prism 8.2*. Para a análise dos dados foram utilizados os testes Mann-Whitney, Kruskal-Wallis e Wilcoxon. Os resultados foram considerados estatisticamente significativos quando $p<0,05$.

REFERÊNCIAS

ABEYAKIRTHI, S.; MOWBRAY, M.; BREDENKAMP, N. *et al*. Arginase is overactive in psoriatic skin. **British Journal of Dermatology**, [s.I.], p.193-196, 17 mar. 2010. Wiley. Disponível em: http://dx.doi.org/10.1111/j.1365-2133.2010.09766.x.

AGGARWAL, S.; GHILARDI, N.; XIE, M. H.; DE SAUVAGE, F. J.; GURNEY, A. L. Interleukin-23 promotes a distinct CD4 T Cell activation state characterized by the production of Interleukin-17. **Journal of Biological Chemistry**, [s.I.], v. 278, n. 3, p.1910-1914, jan. 2003. American Society for Biochemistry & Molecular Biology (ASBMB). Disponível em: http://dx.doi.org/10.1074/jbc.m207577200.

AGGARWAL, S.; GURNEY, A. L.. IL-17: prototype member of an emerging cytokine family. **Journal of the Leokocyte Biology**, [s.I.], v. 1, n. 71, p.1-8, jan. 2002. Disponível em: http://bit.ly/368maHN.

ALLEN, M. H.; VEAL, C.; FAASSEN, A.; POWIS, S. H.; VAUGHAN, R.W.; TREMBATH, R.C. *et al*. A non-HLA gene within the MHC in psoriasis. **The Lancet**, [s.I.], v. 353, n. 9164, p.1589-1590, maio 1999. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/s0140-6736(99)01618-9.

ANBUNATHAN, H.; BOWCOCK, A. M.. The Molecular Revolution in Cutaneous Biology: The Era of Genome-Wide Association Studies and Statistical, Big Data, and Computational Topics. **Journal of Investigative Dermatology**, [s.I.], v. 137, n. 5, p.113-118, maio 2017. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.jid.2016.03.047.

ARICAN, O.; ARAL, M.; SASMAZ, S.; CIRAGIL, P. Serum Levels of TNF-α, IFN-γ, IL-6, IL-8, IL-12, IL-17, and IL-18 in Patients With Active Psoriasis and Correlation With Disease Severity. **Mediators of Inflammation**, [s.I.], v. 2005, n. 5, p.273-279, 2005. Hindawi Limited. Disponível em: http://dx.doi.org/10.1155/mi.2005.273.

ARISAWA, T.; TAHARA, T.; SHIBATA, T.; NAGASAKA, M.; NAKAMURA, M.; KAMIYA, Y. *et al*. The Influence of Polymorphisms of Interleukin-17A and Interleukin-17F Genes on the Susceptibility to Ulcerative Colitis. **Journal Of Clinical Immunology**, [s.I.], v. 28, n. 1, p.44-49, 9 jan. 2008. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1007/s10875-007-9125-8.

ASADULLAH, K.; SABAT, R.; FRIEDRICH, M.; VOLK, H. D.; STERRY, W. Interleukin-10: An Important Immunoregulatory Cytokine With Major Impact on Psoriasis. **Current Drug Target -inflammation & Allergy**, [s.I.], v. 3, n. 2, p.185-192, 1 jun. 2004. Bentham Science Publishers Ltd. Disponível em: http://dx.doi.org/10.2174/1568010043343886.

ASADULLAH, K.; STERRY, W.; STEPHANEK, K.; JASULAITIS, D.; LEUPOLD, M.; AUDRING, H. *et al*. IL-10 is a key cytokine in psoriasis. Proof of principle by IL-10 therapy: a new therapeutic approach. **Journal Of Clinical Investigation**, [s.I.], v. 101, n. 4, p.783-794, 15 fev. 1998. American Society for Clinical Investigation. Disponível em: http://dx.doi.org/10.1172/jci1476.

ASARCH, A.; BARAK, O.; LOO, D. S.; GOTTLIEB, A. B. Th17 cells: A new therapeutic target in inflammatory dermatoses. **Journal of Dermatological Treatment**, [s.I.], v. 19, n. 6, p.318-326, jan. 2008. Informa UK Limited. Disponível em: http://dx.doi.org/10.1080/09546630802206660.

BATALLA, A.; COTO, E.; GONZÁLEZ-LARA, L.; GONZÁLEZ-FERNÁNDEZ, D.; GÓMEZ, J.; ARANGUREN, T. F. *et al*. IL17RA gene variants and anti-TNF response among psoriasis patients. **The Pharmacogenomics Journal**, [s.I.], v. 18, n. 1, p.76-80, 27 set. 2016. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1038/tpj.2016.70.

BATALLA, A.; COTO, E.; GONZÁLEZ-LARA, L.; GONZÁLEZ-FERNÁNDEZ, D.; GÓMEZ, J.; ARANGUREN, T. F. *et al.* Association between single nucleotide polymorphisms IL17RA rs4819554 and IL17E rs79877597 and Psoriasis in a Spanish cohort. **Journal of Dermatological Science**, [s.I.], v. 80, n. 2, p.111-115, nov. 2015. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.jdermsci.2015.06.011.

BÉRARD, F.; NICOLAS, J. F. Physiopathology of psoriasis. **Ann Dermatol Venerol**, [s.I.], v. 130, p.837-842, ago. 2003.

BETTELLI, E.; OUKKA, M.; KUCHROO, V. K. TH-17 cells in the circle of immunity and autoimmunity. **Nature Immunology**, [s.I.], v. 8, n. 4, p.345-350, abr. 2007. Springer Nature. Disponível em: http://dx.doi.org/10.1038/ni0407-345.

BIAłECKA, M.; OSTASZ, R.; KURZAWSKI, M.; KLIMOWICZ, A.; FABIAŃCZYK, H.; BOJKO, P. *et al*. IL17A and IL17F Gene Polymorphism Association with Psoriasis Risk and Response to Treatment in a Polish Population. **Dermatology**, [s.I.], v. 232, n. 5, p.592-596, 2016. S. Karger AG. Disponível em: http://dx.doi.org/10.1159/000448090.

BONIFACE, K.; BERNARD, F. X.; GARCIA, M.; GURNEY, A. L.; LECRON, J. C.; MOREL, F.. IL-22 Inhibits Epidermal Differentiation and Induces Proinflammatory Gene Expression and Migration of Human Keratinocytes. **The Journal of Immunology**, [s.I.], v. 174, n. 6, p.3695-3702, 4 mar. 2005. The American Association of Immunologists. Disponível em: http://dx.doi.org/10.4049/jimmunol.174.6.3695.

BONIFACE, K. *et al*. A role for T cell-derived interleukin 22 in psoriatic skin inflammation. **Clinical & Experimental Immunology**, v. 150, n. 3, p. 407–415, 27 set. 2007. Disponível em: https://doi.org/10.1111/j.1365-2249.2007.03511.x.

BONIFATI, C.; AMEGLIO, F. Cytokines in psoriasis. ***International Journal of Dermatology****, [s.I.], v.38, n.4, p.* 241–251, abr 1999. Wiley. Disponível em: http://dx.doi.org/10.1046/j.1365-4362.1999.00622.x

BOURNERIAS, I.; CHOSIDOW, O. Méthotrexate et psoriasis. Pharmacologie et prise en charge thérapeutique. **Ann Dermatol Venereol**, [s.I.], v.121. n.1, p. 69-74, 1994.

BOWCOCK, A. M.. Genetic association in psoriasis. **Eighth International Psoriasis Genetics Committee Meeting**. Paris, France. 2005. Unpublished.

BRUCH-GERHARZ, D.; SCHNORR, O.; SUSCHEK, C.; BECK, K. F.; PFEILSCHIFTER, J.; RUZICKA, T. *et al*. Arginase 1 Overexpression in Psoriasis. **The**

American Journal of Pathology, [s.I.], v. 162, n. 1, p.203-211, jan. 2003. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/s0002-9440(10)63811-4.

BRUCH-GERHARZ, D. RUZICKA, T., KOLB-BACHOFEN. Nitric Oxide Andits Implication In Skin Homeostais And Disease. Archives of Dermatological Research, v. 290, n.12, p. 643-651, Dez. 1998. Disponível em: https://doi.org/10.1007/s004030050367.

CALDAROLA, G.; DE SIMONE, C.; CARBONE, A.; TULLI, A.; AMERIO, P.; FELICIANI, C.. TNFalpha and its Receptors in Psoriatic Skin, before and after Treatment with Etanercept. **International Journal of Immunopathology and Pharmacology**, [s.I.], v. 22, n. 4, p.961-966, out. 2009. SAGE Publications. Disponível em: http://dx.doi.org/10.1177/039463200902200411.

CAPON, F.; DI MEGLIO, P.; SZAUB, J.; PRESCOTT, N. J.; DUNSTER, C.; BAUMBER, L. *et al*. Sequence variants in the genes for the interleukin-23 receptor (IL23R) and its ligand (IL12B) confer protection against psoriasis. **Human Genetics**, [s.I.], v. 122, n. 2, p.201-206, 22 jun. 2007. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1007/s00439-007-0397-0.

CAPON, F.; MUNRO, M.; BARKER, J.; TREMBATH, R. Searching for the Major Histocompatibility Complex Psoriasis Susceptibility Gene. **Journal of Investigative Dermatology**, [s.I.], v. 118, n. 5, p.745-751, maio 2002. Elsevier BV. Disponível em: http://dx.doi.org/10.1046/j.1523-1747.2002.01749.x.

CHAN, E. S.; CRONSTEIN, B. N.. Molecular action of methotrexate in inflammatory diseases. **Arthritis Research & Therapy**, [s.I.], v. 4, n. 4, p.266-273, 19 mar. 2002. Disponível em: http://bit.ly/31MXzEZ.

CHAN, J. R.; BLUMENSCHEIN, W.; MURPHY, E.; DIVEU, C.; WIEKOWSKI, M.; ABBONDANZO, S. *et al*. IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis.**The Journal of Experimental Medicine**, [s.I.], v. 203, n.12, p. 2557–2587, 30 out. 2006. Rockfeller University Press. Disponível em: http://dx.doi.org/10.1084/jem.20060244.

CHIRICOZZI, A.; GUTTMAN-YASSKY, E.; SUAREZ-FARINAS, M.; NOGRALES, K.E.; TIAN, S.; CARDINALE, I. *et al*. Integrative Responses to IL-17 and TNF-α in Human Keratinocytes Account for Key Inflammatory Pathogenic Circuits in Psoriasis. **Journal of Investigative Dermatology**, [s.I.], v. 131, n. 3, p.677-687, mar. 2011. Elsevier BV. Disponível em: http://dx.doi.org/10.1038/jid.2010.340.

CHIRICOZZI, A.; KRUEGER, J. G.. IL-17 targeted therapies for psoriasis. **Expert Opinion On Investigational Drugs**, [s.I.], v. 22, n. 8, p. 993-1005, 3 jun. 2013. Informa Healthcare. Disponível em: http://dx.doi.org/10.1517/13543784.2013.806483.

CHIRICOZZI, A.; NOGRALES, K. E.; JOHNSON-HUANG, L. M.; FUENTES-DUCULAN, J.; CARDINALE, I.; BONIFACIO, K. M. *et al*. IL-17 Induces an Expanded Range of Downstream Genes in Reconstituted Human Epidermis Model. **Plos One**, [s.I.], v. 9, n. 2, p. e90284, 28 fev. 2014. Public Library of Science (PLoS). Disponível em: http://dx.doi.org/10.1371/journal.pone.0090284.

CHIRICOZZI, A.; ROMANELLI, M.; PANDURI, S.; DONETTI, E.; PRIGNANO, F.. Relevance of in vitro 3-D skin models in dissecting cytokine contribution to psoriasis

pathogenesis. **Histology and Histopathology**, [s.I.], v. 32, p.893-898, sep. 2017. Sercrisma International s.l. Disponível em: http://dx.doi.org/10.14670/HH-11-877.

CHIRICOZZI, A.; ROMANELLI, P.; VOLPE, E.; BORSELLINO, G.; ROMANELLI, M.. Scanning the Immunopathogenesis of Psoriasis. **International Journal of Molecular Sciences**, [s.I.], v. 19, n. 1, p.179, 8 jan. 2018. MDPI AG. Disponível em: http://dx.doi.org/10.3390/ijms19010179.

CHO, M. L.; KANG, J. W.; MOON, Y. M.; NAM, H. J.; JHUN, J. Y.; HEO, S. B. *et al*. STAT3 and NF-kappaB signal pathway is required for IL-23-mediated IL-17 production in spontaneous arthritis animal model IL-1 receptor antagonist-deficient mice. **The Journal of Immunology**, [s.I.], v. 176, n. 9, p.5652-5661, 18 abr. 2006. The American Association of Immunologists. Disponível em: http://dx.doi.org/10.4049/jimmunol.176.9.5652.

CHRISTOPHERS, E. Psoriasis - epidemiology and clinical spectrum. **Clinical and Experimental Dermatology**, [s.I.], v. 26, n. 4, p.314-320, jun. 2001. Wiley. Disponível em: http://dx.doi.org/10.1046/j.1365-2230.2001.00832.x

CROMBEZ, E. A.; CEDERBAUM, S. D. Hyperargininemia due to liver arginase deficiency. **Molecular Genetics and Metabolism**, [s.I.], v. 84, n. 3, p.243-251, mar. 2005. Elsevier BV. http://dx.doi.org/10.1016/j.ymgme.2004.11.004.

CURA, A. J.; CARRUTHERS, A. Role of monosaccharide transport proteins in carbohydrate assimilation, distribution, metabolism, and Homeostasis. **Comprehensive Physiology**, [s.I.], p.863-914, abr. 2012. John Wiley & Sons, Inc. Disponível em: http://dx.doi.org/10.1002/cphy.c110024.

DAHLMAN-GHOZLAN, K.; ORTONNE, J. P.; HEILBORN, J. D.; STEPHANSSON, E.. Altered tissue expression pattern of cell adhesion molecules, ICAM-1, E-selectin and VCAM-1, in bullous pemphigoid during methotrexate therapy. **Experimental Dermatology**, [s.I.], v. 13, n. 2, p.65-69, fev. 2004. Wiley. Disponível em: http://dx.doi.org/10.1111/j.0906-6705.2004.00113.x.

DE CAESTECKER, M. P.; PIEK, E.; ROBERTS, A. B. Role of transforming growth factor-beta signaling in cancer. **Journal of the National Cancer Institute**, [s.I.], v.92, n. 17, p. 1388–1402, 6 set 2000. Oxford University Press (OUP). Disponível em: http://dx.doi.org/10.1093/jnci/92.17.1388.

DI CESARE, A.; DI MEGLIO, P.; NESTLE, F. O. The IL-23/Th17 Axis in the Immunopathogenesis of Psoriasis. **Journal of Investigative Dermatology**, [s.I.], v. 129, n. 6, p.1339-1350, jun. 2009. Elsevier BV. Disponível em: http://dx.doi.org/10.1038/jid.2009.59.

DIERCKX, R. A.; VAN DE WIELE, C. FDG uptake, a surrogate of tumour hypoxia? **European Journal of Nuclear Medicine and Molecular Imaging**, [s.I.], v. 35, n. 8, p.1544-1549, 29 maio 2008. Springer Nature. Disponível em: http://dx.doi.org/10.1007/s00259-008-0758-5.

DIZIKES, G. J.; GRODY, W. W.; KERN, R. M.; CEDERBAUM, S. D. Isolation of human liver arginase cDNA and demonstration of nonhomology between the two human arginase genes. **Biochemical and Biophysical Research Communications**,

[s.I.], v. 141, n. 1, p.53-59, nov. 1986. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/s0006-291x(86)80333-3.

DONETTI, E.; CORNAGHI, L.; ARNABOLDI, F.; LANDONI, F.; ROMAGNOLI, P.; MASTROIANNI, N. *et al*. Interleukin 22 early affects keratinocyte differentiation, but not proliferation, in a three-dimensional model of normal human skin. **Experimental Cell Research**, [s.I.], v. 345, n. 2, p.247-254, jul. 2016. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.yexcr.2016.05.004.

DUDAKOV, J. A.; HANASH, A. M.; VAN DEN BRINK, M. R. M. Interleukin-22: Immunobiology and Pathology. **Annual Review of Immunology**, [s.I.], v. 33, n. 1, p.747-785, 21 mar. 2015. Annual Reviews. Disponível em: http://dx.doi.org/10.1146/annurev-immunol-032414-112123.

DUERR, R. H.; TAYLOR, K. D.; BRANT, S. R.; RIOUX, J. D.; SILVERBERG, M. S.; DALY, M. J. *et al*. A Genome-Wide Association Study Identifies IL23R as an Inflammatory Bowel Disease Gene. **Science**, [s.I.], v. 314, n. 5804, p.1461-1463, 1 dez. 2006. American Association for the Advancement of Science (AAAS). Disponível em: http://dx.doi.org/10.1126/science.1135245.

DUMOUTIER, L.; LEJEUNE, D.; COLAU, D.; RENAULD, J. C.. Cloning and Characterization of IL-22 Binding Protein, a Natural Antagonist of IL-10-Related T Cell-Derived Inducible Factor/IL-22. **The Journal of Immunology**, [s.I.], v. 166, n. 12, p.7090-7095, 15 jun. 2001. The American Association of Immunologists. Disponível em: http://dx.doi.org/10.4049/jimmunol.166.12.7090.

DUVALLET, E.; SEMERANO, L.; ASSIER, E.; FALGARONE, G.; BOISSIER, M. C. Interleukin-23: A key cytokine in inflammatory diseases. **Annals of Medicine**, [s.I.], v. 43, n. 7, p.503-511, 17 maio 2011. Informa UK Limited. Disponível em: http://dx.doi.org/10.3109/07853890.2011.577093.

EFRON, D.; BARBUL, A. Modulation of inflammation and immunity by arginine supplements. **Current Opinion in Clinical Nutrition and Metabolic Care**, [s.I.], v. 6, n. 1, p.531-538, nov. 1998. Disponível em: https://doi.org/10.1097/00075197-199811000-00010.

ELDER, J. T. PSORS1: Linking Genetics and Immunology. **Journal of Investigative Dermatology**, [s.I.], v. 126, n. 6, p.1205-1206, jun. 2006. Elsevier BV. Disponível em: http://dx.doi.org/10.1038/sj.jid.5700357.

ELLIOTT, R. L.; BLOBE, G. C. Role of Transforming Growth Factor Beta in Human Cancer. **Journal of Clinical Oncology**, [s.I.], v. 23, n. 9, p.2078-2093, 20 mar. 2005. American Society of Clinical Oncology (ASCO). Disponível em: http://dx.doi.org/10.1200/jco.2005.02.047.

ELSON, D. A.; RYAN, H. E.; SNOW, J. W.; ARBEIT, J. M.. Coordinate Up-Regulation of Hypoxia Inducible Factor (HIF)-1α and HIF-1 Target Genes during Multi-Stage Epidermal Carcinogenesis and Wound Healing. **Cancer Research**, [s.I.], p.6189-6195, nov. 2000. Disponível em: http://bit.ly/2MRBVvb

ETTEHADI, P.; GREAVES, M. W.; WALLACH, D.; ADERKA, D.; CAMP, R. D. Elevated tumour necrosis factor-alpha (TNF-α) biological activity in psoriatic skin

lesions. **Clinical & Experimental Immunology**, [s.I.], v. 96, n. 1, p.146-151, abr. 1994. Wiley. Disponível em: http://dx.doi.org/10.1111/j.1365-2249.1994.tb06244.x.

FÖRSTERMANN, U.; CLOSS, E. I.; POLLOCK, J. S.; NAKANE, M.; SCHWARZ, P.; GATH, I.; KLEINERT, H. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. **Hypertension**, [s.I.], v. 23, n. 62, p.1121-1131, jun. 1994. Ovid Technologies (Wolters Kluwer Health). Disponível em: http://dx.doi.org/10.1161/01.hyp.23.6.1121.

FOTIADOU, C.; LAZARIDOU, E.; SOTIRIOU, E.; GEROU, S.; KYRGIDIS, A.; VAKIRLIS, E. *et al.* IL-17A, IL-22, and IL-23 as Markers of Psoriasis Activity. **Journal of Cutaneous Medicine and Surgery**, [s.I.], v. 19, n. 6, p.555-560, 27 abr. 2015. SAGE Publications. Disponível em: http://dx.doi.org/10.1177/1203475415584503.

FUJISHIMA, S.; WATANABE, H.; KAWAGUCHI, M.; SUZUKI, T.; MATSUKURA, S.; HOMMA, T. *et al*. Involvement of IL-17F via the induction of IL-6 in psoriasis. **Archives of Dermatological Research**, [s.I.], v. 302, n. 7, p.499-505, 11 fev. 2010. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1007/s00403-010-1033-8.

GAFFEN, S. L. Structure and signalling in the IL-17 receptor family. **Nature Reviews Immunology**, [s.I.], v. 9, n. 8, p.556-567, 3 jul. 2009. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1038/nri2586.

GAFFEN, S. L.; KRAMER, J. M.; YU, J. J.; SHEN, F. The IL-17 Cytokine Family. **Interleukins**, [s.I.], p.255-282, 2006. Elsevier. Disponível em: http://dx.doi.org/10.1016/s0083-6729(06)74010-9.

GARCÍA-DIEZ A. FERRANDIZ FORASTER C. VANACLOCHA SEBASTIÁN F. LIZÁN TUDELA L. BADIA LLACH X. SELLERS FERNÁNDEZ G. What characterizes the severity of psoriasis? Results from an epidemiological study of over 3,300 patients in the Iberian region. **Dermatology**, Basel, v. 216, n. 2, p. 137-151, 2008. Disponível em: https://www.karger.com/Article/Pdf/111511.

GASTL, G. A.; ABRAMS, J. S.; NANUS, D. M.; OOSTERKAMP, R.; SILVER, J.; LIU, F. et al Interleukin-10 production by human carcinoma cell lines and its relationship to interleukin-6 expression. **International Journal of Cancer**, [s.I.], v. 55, n. 1, p.96-101, 19 ago. 1993. Wiley. Disponível em: http://dx.doi.org/10.1002/ijc.2910550118.

GATENBY, R.A.; SMALLBONE, K.; MAINI, P. K.; ROSE, F.; AVERILL, J.; NAGLE, R. B. et al. Cellular adaptations to hypoxia and acidosis during somatic evolution of breast cancer. **British Journal of Cancer**, [s.I.], v. 97, n. 5, p.646-653, ago. 2007. Springer Nature. Disponível em: http://dx.doi.org/10.1038/sj.bjc.6603922.

GHERZI, R.; MELIOLI, G.; DE LUCA, M.; D'AGOSTINO, A.; DISTEFANO, A.; GUASTELLA, M. *et al*. "HepG2/erythroid/brain" type glucose transporter (GLUT1) is highly expressed in human epidermis: Keratinocyte differentiation affects GLUT1 levels in reconstituted epidermis. **Journal of Cellular Physiology**, [s.I.], v. 150, n. 3, p.463-474, mar. 1992. Wiley. Disponível em: http://dx.doi.org/10.1002/jcp.1041500306.

GOMI, T.; SHIOHARA, T.; MUNAKATA, T.; IMANISHI, K.; NAGASHIMA, M.. Interleukin 1α, tumor necrosis factor α, and interferon γ in psoriasis. **Arch Dermatol.**, [s.I.], v. 127, p.827-830, jun. 1991. Disponível em: doi:10.1001/archderm.1991.01680050071006.

GOTOH, T.; ARAKI, M.; MORI, M. Chromosomal Localization of the Human Arginase II Gene and Tissue Distribution of Its mRNA. **Biochemical and Biophysical Research Communications**, [s.I.], v. 233, n. 2, p.487-491, abr. 1997. Elsevier BV. Disponível em: http://dx.doi.org/10.1006/bbrc.1997.6473.

GOTOH, T.; SONOKI, T.; NAGASAKI, A.; TERADA, K.; TAKIGUCHI, M.; MORI, M.. Molecular cloning of cDNA for nonhepatic mitochondrial arginase (arginase II) and comparison of its induction with nitric oxide synthase in a murine macrophage-like cell line. **Febs Letters**, [s.I.], v. 395, n. 2-3, p.119-122, 21 out. 1996. Wiley. Disponível em: http://dx.doi.org/10.1016/0014-5793(96)01015-0.

GOURRAUD, P. A.; KHANKHANIAN, P.; CEREB, N.; YANG, S. Y.; FEOLO, M.; MAIERS, M. *et al*. HLA Diversity in the 1000 Genomes Dataset. **Plos One**, [s.I.], v. 9, n. 7, p.e97282, 2 jul. 2014. Public Library of Science (PLoS). Disponível em: http://dx.doi.org/10.1371/journal.pone.0097282.

GREB, J. E.; GOLDMINZ, A. M.; ELDER, J. T.; LEBWOHL, M. G.; GLADMANN, D. D.; WU, J. J. *et al*. Psoriasis. **Nature Reviews Disease Primers**, [s.I.], v. 2, n. 1, p.1-7, 24 nov. 2016. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1038/nrdp.2016.82.

GRIFFITHS, C. E.; BARKER, J. N. Pathogenesis and clinical features of psoriasis. **The Lancet**, [s.I.], v. 370, n. 9583, p.263-271, jul. 2007. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/s0140-6736(07)61128-3.

GUDJONSSON, J. E.; JOHNSTON, A.; SIGMUNDSDOTTIR, H; VALDIMARSSON, H.. Immunopathogenic mechanisms in psoriasis. **Clinical and Experimental Immunology**, [s.I.], v. 135, n. 1, p.1-8, jan. 2004. Wiley. Disponível em: http://dx.doi.org/10.1111/j.1365-2249.2004.02310.x.

GUILLOTEAU, K.; PARIS, I.; PEDRETTI, N.; BONIFACE, K.; JUCHAUX, F.; HUGUIER, V. *et al.* Skin Inflammation Induced by the Synergistic Action of IL-17A, IL-22, Oncostatin M, IL-1α, and TNF-α Recapitulates Some Features of Psoriasis. **The Journal of Immunology**, [s.I.], v. 184, n. 9, p.5263-5270, 24 mar. 2010. The American Association of Immunologists. Disponível em: http://dx.doi.org/10.4049/jimmunol.0902464.

HARPER, E. G.; GUO, C.; RIZZO, H.; LILLIS, J. V.; KURTZ, S. E.; SKORCHEVA, I. *et al*. Th17 Cytokines Stimulate CCL20 Expression in Keratinocytes In Vitro and In Vivo: Implications for Psoriasis Pathogenesis. **Journal of Investigative Dermatology**, [s.I.], v. 129, n. 9, p.2175-2183, set. 2009. Elsevier BV. Disponível em: http://dx.doi.org/10.1038/jid.2009.65.

HARRINGTON, L.; HATTON, R. D.; MANGAN, P. R.; TURNER, H.; MURPHY, T. L.; MURPHY, K.M. *et al*. Interleukin 17–producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. **Nature Immunology**, [s.I.],

v. 6, n. 11, p.1123-1132, 2 out. 2005. Springer Nature. Disponível em: http://dx.doi.org/10.1038/ni1254.

HE, W.; DORN, D. C.; ERDJUMENT-BROMAGE, H.; TEMPST, P.; MOORE, M. A.; MASSAGUÉ, J.. Hematopoiesis Controlled by Distinct TIF1γ and Smad4 Branches of the TGFβ Pathway. **Cell**, [s.I.], v. 125, n. 5, p.929-941, jun. 2006. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.cell.2006.03.045.

HE, X. C.; ZHANG, J.; TONG, W. G.; TAWFIK, O.; ROSS, J.; SCOVILLE, D. H. *et al.* BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt–β-catenin signaling. **Nature Genetics**, [s.I.], v. 36, n. 10, p.1117-1121, 19 set. 2004. Disponível em: Springer Nature. http://dx.doi.org/10.1038/ng1430.

HERMAN, S.; ZURGIL, N.; DEUTSCH, M.. Low dose methotrexate induces apoptosis with reactive oxygen species involvement in T lymphocytic cell lines to a greater extent than in monocytic lines. **Inflammation Research**, [s.I.], v. 54, n. 7, p.273-280, jul. 2005. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1007/s00011-005-1355-8.

HODEIB, A. A.; NEINAA, Y. M. E.; ZAKARIA, S. S.; ALSHENAWY, H. A.. Glucose transporter-1 (GLUT-1) expression in psoriasis: correlation with disease severity. **International Journal of Dermatology**, [s.I.], v. 57, n. 8, p.943-951, 24 maio 2018. Wiley. Disponível em: http://dx.doi.org/10.1111/ijd.14037.

JENKINSON, C. P.; GRODY, W. W.; CEDERBAUM, S. D. Comparative properties of arginases. **Comparative Biochemistry and Physiology Part B**: Biochemistry and Molecular Biology, [s.I.], v. 114, n. 1, p.107-132, maio 1996. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/0305-0491(95)02138-8.

JIA, L.; WU, C.. The Biology and Functions of Th22 Cells. **Advances in Experimental Medicine and Biology**, [s.I.], p.209-230, 2014. Springer Netherlands. Disponível em: http://dx.doi.org/10.1007/978-94-017-9487-9_8.

JOOST, H.-G.; BELL, G. I.; BEST, J. D.; BIRNBAUM, M. J.; CHARRON, M. J.; CHEN, Y. T. *et al*. Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. **American Journal of Physiology-Endocrinology and Metabolism**, [s.I.], v. 282, n. 4, p.974-976, 1 abr. 2002. American Physiological Society. Disponível em: http://dx.doi.org/10.1152/ajpendo.00407.2001.

KAGAMI, S.; RIZZO, H. L.; LEE, J. J.; KOGUCHI, Y.; BLAUVELT, A. Circulating Th17, Th22, and Th1 Cells Are Increased in Psoriasis. **Journal of Investigative Dermatology**, [s.I.], v. 130, n. 5, p.1373-1383, dez. 2009. Elsevier BV. Disponível em: http://dx.doi.org/10.1038/jid.2009.399.

KARAM, R. A.; ZIDAN, H. E.; KHATER, M. H. Polymorphisms in the TNF-α and IL-10 gene promoters and risk of psoriasis and correlation with disease severity. **Cytokine**, [s.I.], v. 66, n. 2, p.101-105, abr. 2014. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.cyto.2014.01.008.

KAWAGUCHI, M.; ADACHI, M.; ODA, N.; KOKUBU, F.; HUANG, S. K. IL-17 cytokine family. **Journal of Allergy and Clinical Immunology**, [s.I.], v. 114, n. 6, p.1265-1273, dez. 2004. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.jaci.2004.10.019.

KIM, J. H.; BUGAJ, L. J.; OH, Y. J.; BIVALACQUA, T. J.; RYOO, S.; SOUCY, K. G. *et al*. Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats. **Journal of Applied Physiology**, [s.I.], v. 107, n. 4, p.1249-1257, out. 2009. American Physiological Society. Disponível em: http://dx.doi.org/10.1152/japplphysiol.91393.2008.

KIMURA, A.; NAKA, T.; KISHIMOTO, T. IL-6-dependent and -independent pathways in the development of interleukin 17-producing T helper cells. **Proceedings of the National Academy of Sciences**, [s.I.], v. 104, n. 29, p.12099-12104, 10 jul. 2007. Proceedings of the National Academy of Sciences. Disponível em: http://dx.doi.org/10.1073/pnas.0705268104

KOGA, C.; KABASHIMA, K.; SHIRAISHI, N.; KOBAYASHI, M.; TOKURA, Y. Possible Pathogenic Role of Th17 Cells for Atopic Dermatitis. **Journal of Investigative Dermatology**, [s.I.], v. 128, n. 11, p.2625-2630, nov. 2008. Elsevier BV. Disponível em: http://dx.doi.org/10.1038/jid.2008.111.

KRISTENSEN, M.; CHU, C. Q.; EEDY, D. J.; FELDMANN, M.; BRENNAN, F. M.; BREATHNACH, S. M.. Localization of tumour necrosis factor-alpha (TNF-alpha) and its receptors in normal and psoriatic skin: epidermal cells express the 55-kD but not the 75-kD TNF receptor. **Clinical & Experimental Immunology**, [s.I.], v. 94, n. 2, p.354-362, nov. 1993. Wiley. Disponível em: http://dx.doi.org/10.1111/j.1365 2249.1993.tb03457.x.

KRÖNCKE, K. D.; FEHSEL, K.; KOLB-BACHOFEN, V. Nitric Oxide: Cytotoxicity versus Cytoprotection— How, Why, When, and Where?. **Nitric Oxide**, [s.I.], v. 1, n. 2, p.107-120, abr. 1997. Elsevier BV. Disponível em: http://dx.doi.org/10.1006/niox.1997.0118.

KRUEGER, G.; ELLIS, C. N. Psoriasis—recent advances in understanding its pathogenesis and treatment. **Journal of the American Academy of Dermatology**, [s.I.], v. 53, n. 1, p.94-100, jul. 2005. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.jaad.2005.04.035.

KURTOVIC, N. O.; HALILOVIC, E. K. Serum Concentrations of Interferon Gamma (IFN- γ) in Patients with Psoriasis: Correlation with Clinical Type and Severity of the Disease. **Medical Archives**, [s.I.], v. 72, n. 6, p.410-413, 2018. ScopeMed International Medical Journal Management and Indexing System. Disponível em: http://dx.doi.org/10.5455/medarh.2018.72.410-413.

LANDE, R.; GREGORIO, J.; FACCHINETTI, V.; CHATTERJEE, B.; WANG, Y. H.; HOMEY, B. *et al.* Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. **Nature**, [s.I.], v. 449, n. 7162, p.564-569, 16 set. 2007. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1038/nature06116.

LANGRISH, C. L.; CHEN, Y.; BLUMENSCHEIN, W. M.; MATTSON, J.; BASHAM, B.; SEDGWICK, J. D. *et al.* IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. **The Journal of Experimental Medicine**, [s.I.], v. 201, n. 2, p.233-240, 17 jan. 2005. Rockefeller University Press. Disponível em: http://dx.doi.org/10.1084/jem.20041257.

LEE, E.; TREPICCHIO, W. L.; OESTREICHER, J. L.; PITTMAN, D.; WANG, F.; CHAMIAN, F. *et al.* Increased Expression of Interleukin 23 p19 and p40 in Lesional Skin of Patients with Psoriasis Vulgaris. **The Journal of Experimental Medicine**, [s.I.], v. 199, n. 1, p.125-130, 5 jan. 2004. Rockefeller University Press. Disponível em: http://dx.doi.org/10.1084/jem.20030451.

LI, J.; CHEN, X.; LIU, Z.; YUE, Q.; LIU, H. Expression of Th17 cytokines in skin lesions of patients with psoriasis. **Journal of Huazhong University of Science and Technology**, [s.I.], v. 27, n. 3, p.330-332, jun. 2007. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1007/s11596-007-0329-1.

LIEW, F.Y.; COX, F.E.G. Nonspecific defense mechanism: the role of nitric oxide. **Immunology Today**, [s.I.], v. 12, n. 3, p. 17-21, jan. 1991. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/s0167-5699(05)80006-4.

LIMA, H. C.. Papel das células T reguladoras no desenvolvimento de dermatoses. **Anais Brasileiros de Dermatologia**, [s.I.], v. 3, n. 81, p.269-281, 2006. Disponível em: http://dx.doi.org/10.1590/S0365-05962006000300010.

LIU, Y.; KRUEGER, J. G.; BOWCOCK, A. M.. Psoriasis: genetic associations and immune system changes. **Genes & Immunity**, [s.I.], v. 8, n. 1, p.1-12, 9, 2007. Springer Nature. Disponível em: http://dx.doi.org/10.1038/sj.gene.6364351.

LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T) Method. **Methods,** v. 25, n. 4, p. 402-8, Dec 2001. Disponível em: https://doi.org/10.1006/meth.2001.1262.

LØNNBERG, A. S.; ZACHARIAE, C.; SKOV, L. Targeting of interleukin-17 in the treatment of psoriasis. **Clinical, Cosmetic and Investigational Dermatology**, [s.I.], p.251-259, set. 2014. Dove Medical Press Ltd.. Disponível em: http://dx.doi.org/10.2147/ccid.s67534.

LOWES, M. A.; KIKUCHI, T.; FUENTES-DUCULAN, J.; CARDINALE, I.; ZABA, L. C.; HAIDER, A. S. *et al*. Psoriasis Vulgaris Lesions Contain Discrete Populations of Th1 and Th17 T Cells. **Journal of Investigative Dermatology**, [s.I.], v. 128, n. 5, p.1207-1211, maio 2008. Elsevier BV. Disponível em: http://dx.doi.org/10.1038/sj.jid.5701213.

LOWES, M. A.; RUSSELL, C. B.; MARTIN, D. A.; TOWNE, J. E.; KRUEGER, J. G.. The IL-23/T17 pathogenic axis in psoriasis is amplified by keratinocyte responses. **Trends Immunology**, [s.I.], v. 34, n. 4, p. 174-181, abr. 2013. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.it.2012.11.005.

LOWES, M. A.; SUÁREZ-FARIÑAS, M.; KRUEGER, J. G. Immunology of Psoriasis. **Annual Review of Immunology**, [s.I.], v. 32, n. 1, p.227-255, 21 mar. 2014. Annual Reviews. Disponível em: http://dx.doi.org/10.1146/annurev-immunol-032713-120225.

LYNDE, C. W.; POULIN, Y.; VENDER R.; BOURCIER, M.; KHALIL, S... Interleukin 17A: Toward a new understanding of psoriasis pathogenesis. **Journal of The American Academy of Dermatology**, [s.I.], v. 71, n. 1, p.141-150, jul. 2014. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.jaad.2013.12.036.

MACHADO-PINTO, J.; DINIZ, M. S.; BAVOSO, N. C. Psoriasis: new comorbidities. **Anais Brasileiros de Dermatologia**, Rio de Janeiro, v. 91, n. 1, p. 8-14, jan./fev. 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4782640/pdf/abd-91-01-0008.pdf.

MAHIL, S. K.; CAPON, F.; BARKER, J. N. Update on psoriasis immunopathogenesis and targeted immunotherapy. **Seminars In Immunopathology**, [s.l.], v. 38, n. 1, p.11-27, 16 nov. 2016. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1007/s00281-015-0539-8.

MALAKOUTI, M.; BROWN, G. E.; WANG, E.; KOO, J.; LEVIN, E. C. The role of IL-17 in psoriasis. **Journal of Dermatological Treatment**, [s.I.], v. 26, n. 1, p.41-44, 20 fev. 2014. Informa UK Limited. Disponível em: http://dx.doi.org/10.3109/09546634.2013.879093.

MARQUES, R. G. M.; MAYER-DA-SILVA, A.; FILIPE, L.P**.. Dermatologia:** Ficheiro Clínico e Terapêutico, 1ª ed.. Lisboa, Portugal: Fundação Calouste Gulbenkian, 2010.

MARTIN, D. A.; TOWNE, J. E.; KRICORIAN, G.; KLEKOTKA, P.; GUDIONSSON, J. E.; KRUEGER, J. G. *et al.* The Emerging Role of IL-17 in the Pathogenesis of Psoriasis: Preclinical and Clinical Findings. **Journal of Investigative Dermatology**, [s.I.], v. 133, n. 1, p.17-26, jan. 2013. Elsevier BV. Disponível em: http://dx.doi.org/10.1038/jid.2012.194.

MASSAGUÉ, J.. TGF- β in Cancer. **Cell**, [s.I.], v. 134, n. 2, p.215-230, jul. 2008. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.cell.2008.07.001.

McGEACHY, M. J.; BAK-JENSEN, K. S.; CHEN, Y.; TATO, C. M.; BLUMENSCHEIN, W.; McCLANAHAN, T. *et al.* TGF-β and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain TH-17 cell–mediated pathology. **Nature Immunology**, [s.I.], v. 8, n. 12, p.1390-1397, 11 nov. 2007. Springer Nature. Disponível em: http://dx.doi.org/10.1038/ni1539.

MORI, M.; GOTOH, T. Relationship between arginase activity and Nitric Oxide production. **Nitric Oxide:** Biology and Pathobiology, [San Diego], p.199-208, 2000. Elsevier. Disponível em: http://dx.doi.org/10.1016/b978-012370420-7/50013-7.

MUECKLER, M.; CARUSO, C.; BALDWIN, S. A.; PANICO, M.; BLENCH, I.; MORRIS, H. R. *et al*. Sequence and structure of a human glucose transporter. **Science**, [s.I.], v. 229, n. 4717, p.941-945, 6 set. 1985. American Association for the Advancement of Science (AAAS). Disponível em: http://dx.doi.org/10.1126/science.3839598.

NESTLE, F. O.; CONRAD, C.; TUN-KYI, A.; HOMEY, B.; GOMBERT, M.; BOYMAN, O. *et al*. Plasmacytoid predendritic cells initiate psoriasis through interferon-α production. **The Journal of Experimental Medicine**, [s.I.], v. 202, n. 1,

p.135-143, 4 jul. 2005. Rockefeller University Press. Disponível em: http://dx.doi.org/10.1084/jem.20050500.

NOGRALES, K. E.; ZABA, L. C.; GUTTMAN-YASSKY, E.; FUENTES-DUCULAN, J.; SUÁREZ-FARIÑAS, M.; CARDINALE, I. *et al*. Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. **British Journal of Dermatology**, [s.I.], p.1092-1102, ago. 2008. Wiley. Disponível em: http://dx.doi.org/10.1111/j.1365-2133.2008.08769.x.

O'GARRA, A.; BARRAT, F. J.; CASTRO, A. G.; VICARI, A.; HAWRYLOWICZ, C. Strategies for use of IL-10 or its antagonists in human disease. **Immunological Reviews**, [s.I.], v. 223, n. 1, p.114-131, jun. 2008. Wiley. Disponível em: http://dx.doi.org/10.1111/j.1600-065x.2008.00635.x.

OVIGNE, J. M.; BAKER, B. S.; BROWN, D. W.; POWLES, A. V.; FRY, L.. Epidermal CD8+ T cells in chronic plaque psoriasis are Tc1 cells producing heterogeneous levels of interferon-gamma. **Experimental Dermatology**, [s.I.], v. 10, n. 3, p.168-174, jun. 2001. Wiley. Disponível em: http://dx.doi.org/10.1034/j.1600-0625.2001.010003168.x .

PARK, H.; LI, Z.; YANG, X. O.; CHANG, S. H.; NURIEVA, R.; WANG, Y. H. et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. **Nature Immunology**, [s.I.], v. 6, n. 11, p.1133-1141, 2 out. 2005. Springer Nature. Disponível em: http://dx.doi.org/10.1038/ni1261.

PANTELYUSHIN, S., HAAK, S., INGOLD, B., KULIG, P., HEPPNER, F. L., NAVARINI, A. A., & BECHER, B. Rorγt+ innate lymphocytes and γδ T cells initiate psoriasiform plaque formation in mice. **J Clin Invest**, v. 122, n. 6, p. 2252-6, Jun 2012. ISSN 1558-8238. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/22546855.

PATHIRANA, D.; ORMEROD, A. D.; SAIAG, P.; SMITH, C.; SPULS, P. I.; NAST, A. *et al.* European S3-Guidelines on the systemic treatment of psoriasis vulgaris. **Journal of the Europeans Acadademy of Dermatology and Venereology**, [s.I.], v. 23, p.1-70, out. 2009. Wiley. Disponível em: http://dx.doi,org/10.1111/j.1468-3083.2009.03389.x.

PISA, P.; HALAPI, E.; PISA, E. K.; GERDIN, E.; HISING, C.; BUCHT, A. *et al*. Selective expression of interleukin 10, interferon-gamma, and granulocyte-macrophage colony-stimulating factor in ovarian cancer biopsies. **Proceedings of the National Academy of Sciences**, [s.I.], v. 89, n. 16, p.7708-7712, 15 ago. 1992. Proceedings of the National Academy of Sciences. Disponível em: http://dx.doi.org/10.1073/pnas.89.16.7708

PISKIN, G.; SYLVA-STEENLAND, R. M.; BOS, J. D.; TEUNISSEN, M. B. In vitro and *in situ* expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: Enhanced expression in psoriatic skin. **The Journal of Immunology**, [s.I.], v. 176,n. 3, p.1908-1915, 19 jan. 2006. The American Association of Immunologists. Disponível em: http://dx.doi.org/10.4049/jimmunol.176.3.1908.

PRIETO-PÉREZ, R.; SOLANO-LÓPEZ, G.; CABALEIRO, T.; ROMÁN, M.; OCHOA, D.; TALEGÓN, M. *et al*. The polymorphism rs763780 in the IL-17F gene is associated with response to biological drugs in patients with

psoriasis. **Pharmacogenomics**, [s.I.], v. 16, n. 15, p.1723-1731, out. 2015. Future Medicine Ltd. Disponível em: http://dx.doi.org/10.2217/pgs.15.107.

PRIGNANO, F.; ARNABOLDI, F.; CORNAGHI, L.; LANDONI, F.; TRIPO, L.; BARUFFALDI PREIS, F. W. *et al.* Tumor necrosis factor-alpha and interleukin-17 differently affects Langerhans cell distribution and activation in an innovative three-dimensional model of normal human skin. **European Journal of Cell Biology**, [s.I.], v. 94, n. 2, p.71-77, fev. 2015. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.ejcb.2014.12.003.

PUIG, L.; CARRASCOSA, J. M.; DAUDÉN, E.; SÁNCHEZ-CARAZO, J. L.; FERRÁNDIZ, C.; SÁNCHEZ-REGAÑA, M. *et al*. Spanish evidence-based guidelines on the treatment of moderate-to-severe psoriasis with biologic agents. **Actas Dermosifiliogr**, [s.I.], v. 100, n. 5, p.386-413, jun. 2009. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/19558918.

RIVAS BEJARANO, J. J.; VALDECANTOS, W. C.. Psoriasis as Autoinflammatory Disease. **Dermatologic Clinics**, [s.I.], v. 31, n. 3, p.445-460, jul. 2013. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.det.2013.04.009.

ROBERTS, A. B.; SPORN, M. B. Differential expression of the TGF-beta isoforms in embryogenesis suggests specific roles in developing and adult tissues. **Molecular Reproduction and Development**, [s.I.], v. 32, n. 2, p.91-98, jun. 1992. Wiley. Disponível em: http://dx.doi.org/10.1002/mrd.1080320203.

ROOSTAEYAN, O.; KIVELEVITCH, D.; MENTER, A. A review article on brodalumab in the treatment of moderate-to-severe plaque psoriasis. **Immunotherapy**, [s.I.], v. 9, n. 12, p.963-978, set. 2017. Future Medicine Ltd. Disponível em: http://dx.doi.org/10.2217/imt-2017-0077.

RYAN, S. Psoriasis: characteristics, psychosocial effects and treatment options. **British Journal of Nursing**, [s.I.], v. 17, n. 5, p.284-290, mar. 2008. Mark Allen Group. Disponível em: http://dx.doi.org/10.12968/bjon.2008.17.5.28823.

SABAT, R.; OUYANG, W.; WOLK, K. Therapeutic opportunities of the IL-22–IL-22R1 system. **Nature Reviews Drug Discovery**, [s.I.], v. 13, n. 1, p.21-38, 31 dez. 2014. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1038/nrd4176.

SALEH, A.; ABUHILAL, M.; CHEUNG, B.; Methotrexate in psoriasis: from A to Z, **Journal of Turkish of Academy Dermatology**, [s.I.], v. 4, n. 1, p. 1-13, 2010. Disponível em: http://jtad.org/2010/1/jtad04101r.pdf.

SALVEMINI, D.; MARINO, M. H. Inducible nitric oxide synthase and inflammation. **Expert Opinion on Investigational Drugs**, [s.l.], v. 7, n. 1, p.65-75, jan. 1998. Informa Healthcare. Disponível em: http://dx.doi.org/10.1517/13543784.7.1.65.

SANCHEZ, A. P. G,. Imunopatogênese da psoríase. **Anais Brasileiros de Dermatologia**, [s.I.], v. 85, n. 5, p.747-749, out. 2010. FapUNIFESP (SciELO). Disponível em: http://dx.doi.org/10.1590/s0365-05962010000500028.

SAUNDERS, N.; JETTEN, A. Control of growth regulatory and differentiation-specific genes in human epidermal keratinocytes by interferon gamma. Antagonism by retinoic acid and transforming growth factor beta 1. **The Journal of Biological Chemistry**, [s.I.], v. 269, n. 3, p.2016-2022, 21 jan. 1994. Disponível em: http://www.jbc.org/content/269/3/2016.long.

SCHÖN, M. P.; BOEHNCKE, W. H. Psoriasis. **The New England Journal of Medicine,** Boston, v. 352, n. 18, p. 1899-1912, maio 2005. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/15872205.

SPARKES, R. S.; DIZIKES, G. J.; KLISAK, I.; GRODY, W. W.; MOHANDAS, T.; HEINZMANN, C. ZOLLMAN, S., LUSIS, AJ., CEDERBAUM, SD. The gene for human liver arginase (ARG1) is assigned to chromosome band 6q23. **The American Journal of Human Genetics**, [s.I.], v. 39, n. 2, p.186-193, ago. 1986. Disponível em: http://bit.ly/2WiHvtr.

SRAMEK, M.; NERADIL, J.; VESELSKA, R.. Much more than you expected: The non-DHFR-mediated effects of methotrexate. **Biochimica et Biophysica Acta (bba) - General Subjects**, [s.I.], v. 1861, n. 3, p.499-503, mar. 2017. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.bbagen.2016.12.014.

STARNES, T.; ROBERTSON, M. J.; SLEDGE, G.; KELICH, S.; NAKSHATRI, H.; BROXMEYER, H. E. *et al*. Cutting Edge: IL-17F, a Novel Cytokine Selectively Expressed in Activated T Cells and Monocytes, Regulates Angiogenesis and Endothelial Cell Cytokine Production. **The Journal of Immunology**, [s.I.], v. 167, n. 8, p.4137-4140, 15 out. 2001. The American Association of Immunologists. Disponível em: http://dx.doi.org/10.4049/jimmunol.167.8.4137.

SZEGEDI, A.; ALEKSZA, M.; GONAD, A.; IRINVI, B.; SIPKA, S.; HUYADI, J. *et al.* Elevated rate of Thelper1 (TH1) lymphocytes and serum IFN-γ levels in psoriatic patients. **Immunology Letters**, [s.I.], v. 86, n. 3, p.277-280, maio 2003. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/s0165-2478(03)00025-7.

TAN, J. K.; APHALE, A.; MALAVIYA , R.; YVONNE , S.; GOTTLIEB, A. B.. Mechanisms of Action of Etanercept in Psoriasis. **Journal of Investigative Dermatology Symposium Proceedings**, [s.I.], v. 12, n. 1, p.38-45, maio 2007. Elsevier BV. Disponível em: http://dx.doi.org/10.1038/sj.jidsymp.5650037.

TAO, J.; YANG, J.; WANG, L.; LI, L.; WEN, X.; SHEN, G. X. *et al*. Expression of GLUT-1 in psoriasis and the relationship between GLUT-1 upregulation induced by hypoxia and proliferation of keratinocyte growth. **Journal of Dermatological Science**, [s.I.], v. 51, n. 3, p.203-207, set. 2008. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.jdermsci.2008.04.012.

TEUNISSEN, M. B.; KOOMEN, C. W.; DE WAAL MALEFYT, R.; WIERENGA, E. A.; BOS, J. D. Interleukin-17 and Interferon-γ Synergize in the Enhancement of Proinflammatory Cytokine Production by Human Keratinocytes. **Journal of Investigative Dermatology**, [s.I.], v. 111, n. 4, p.645-649, out. 1998. Elsevier BV. Disponível em: http://dx.doi.org/10.1046/j.1523-1747.1998.00347.x.

TOCHIO, T.; TANAKA, H.; NAKATA, S.. Glucose transporter member 1 is involved in UVB-induced epidermal hyperplasia by enhancing proliferation in epidermal

keratinocytes. **International Journal of Dermatology**, [s.I.], v. 52, n. 3, p.300-308, 20 jan. 2013. Wiley. Disponível em: http://dx.doi.org/10.1111/j.1365-4632.2011.05299.x.

TRAUB, M.; MARSHALL, K.. Psoriasis – Pathophysiology, Conventional, and Alternative Approaches to Treatment. **Alternative Medicine Review**, [s.I.], v. 12, n. 4, p.319-330, dez. 2007. Disponível em: http://bit.ly/2WkxAnl.

TRIFUNOVIĆ, J.; MILLER, L.; DEBELJAK, Ž.; HORVAT, V.. Pathologic patterns of interleukin 10 expression – A review. **Biochemia Medica**, [s.I.], p.36-48, 2015. Croatian Society for Medical Biochemistry and Laboratory Medicine. Disponível em: http://dx.doi.org/10.11613/bm.2015.004.

TSOI, L. C.; SPAIN, S. L.; KNIGHT, J.; ELLINGHAUS, E.; STUART, P. E.; CAPON, F.; *et al.* Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. **Nature Genetics**, [s.I.], v. 44, n. 12, p.1341-1348, 11 nov. 2012. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1038/ng.2467.

WAWRZYCKI, B.; PIETRZAK, A.; GRYWALSKA, E.; KRASOWSKA, D.; CHODOROWSKA, G.; ROLIŃSKI, J. Interleukin-22 and Its Correlation with Disease Activity in Plaque Psoriasis. **Archivum Immunologiae et Therapiae Experimentalis**, [s.I.], v. 67, n. 2, p.103-108, 5 out. 2019. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1007/s00005-018-0527-5.

WEINER, M. F.; MIRANDA, R. N.; BARDALES, R. H.; MUKUNYADZI, P.; BAKER, S. J.; KOROURIAN, S. *et al*. Diagnostic value of GLUT-1 immunoreactivity to distinguish benign from malignant cystic squamous lesions of the head and neck in fine-needle aspiration biopsy material. **Diagnostic Cytopathology**, [s.I.], v. 31, n. 5, p.294-299, 2004. Wiley. Disponível em: http://dx.doi.org/10.1002/dc.20127.

WEINSTEIN, G. D.; JEFFES, E.; MCCULLOUGH, J. L.. Cytotoxic and Immunologic Effects of Methotrexate in Psoriasis. **Journal of Investigative Dermatology**, [s.I.], v. 95, n. 5, p.49-52, nov. 1990. Elsevier BV. Disponível em: http://dx.doi.org/10.1111/1523-1747.ep12505777.

WIEKOWSKI, M. T. ; LEACH, M. W.; EVANS, E. W.; SULLIVAN, L.; CHEN, S. C.; VASSILEVA, G. *et al*. Ubiquitous Transgenic Expression of the IL-23 Subunit p19 Induces Multiorgan Inflammation, Runting, Infertility, and Premature Death. **The Journal of Immunology**, [s.I.], v. 166, n. 12, p.7563-7570, 15 jun. 2001. The American Association of Immunologists. Disponível em: http://dx.doi.org/10.4049/jimmunol.166.12.7563.

WILSON, N. J.; BONIFACE, K.; CHAN, J. R.; McKENZIE, B. S.; BLUMENSCHEIN, W. M.; MATTSON, J. D. *et al.* Development, cytokine profile and function of human interleukin 17–producing helper T cells. **Nature Immunology**, [s.I.], v. 8, n. 9, p.950-957, 5 ago. 2007. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1038/ni1497.

WINTERFIELD, L. S.; MENTER, A.. Infliximab. **Dermatologic Therapy**, [s.I.], v. 17, n. 5, p.409-426, out. 2004. Wiley. Disponível em: http://dx.doi.org/10.1111/j.1396-0296.2004.04044.x.

WITTE, M. B.; BARBUL, A. Role of nitric oxide in wound repair. **The American Journal of Surgery**, [s.I.], v. 183, n. 4, p.406-412, abr. 2002. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/s0002-9610(02)00815-2.

WOLK, K.; HAUGEN, H. S.; XU, W.; WITTE, E.; WAGGIE, K.; ANDERSON, M. et al. IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-γ are not. **Journal of Molecular Medicine**, [s.I.], v. 87, n. 5, p.523-536, 30 mar. 2009. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1007/s00109-009-0457-0.

WOLK, K.; KUNZ, S.; ASADULLAH, K.; SABAT, R. Cutting Edge: Immune Cells as Sources and Targets of the IL-10 Family Members?. **The Journal of Immunology**, [s.I.], v. 168, n. 11, p.5397-5402, 1 jun. 2002. The American Association of Immunologists. Disponível em: http://dx.doi.org/10.4049/jimmunol.168.11.5397.

WOLK, K.; KUNZ, S.; WITTE, E.; FRIEDRICH, M.; ASADULLAH, K.; SABAT, R.. IL-22 Increases the Innate Immunity of Tissues. **Immunity**, [s.I.], v. 21, n. 2, p.241-254, ago. 2004. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.immuni.2004.07.007.

WOLK, K.; WITTE, E.; WALLACE, E.; DOCKE, W. D.; KUNZ, S.; ASADULLAH, K. et al. IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. **European Journal of Immunology**. [s.I.], v. 36, n.5, p.1309-1323, maio 2006. Wiley. Disponível em: http://dx.doi.org/10.1002/eji.200535503.

WOLLINA, U.; HANSEL, G.; KOCH, A.; SCHÖNLEBE, J.; KÖSTLER, E.; HAROSKE, G.. Tumor Necrosis Factor-α Inhibitor-Induced Psoriasis or Psoriasiform Exanthemata. **American Journal of Clinical Dermatology**, [s.I.], v. 9, n. 1, p.1-14, 2008. Springer Nature. Disponível em: http://dx.doi.org/10.2165/00128071-200809010-00001.

WUEPPER, K. D.; COULTER, S. N.; HABERMAN, A.. Psoriasis Vulgaris: A Genetic Approach. **Journal of Investigative Dermatology**, [s.I.], v. 95, n. 5, p.2-4, nov. 1990. Elsevier BV. Disponível em: http://dx.doi.org/10.1111/1523-1747.ep12505638.

XIA, Y.; DAWSON, V. L.; DAWSON, T. M.; SNYDER, S. H.; ZWEIER, J. L. Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. **Proceedings of the National Academy of Sciences**, [s.I.], v. 93, n. 13, p.6770-6774, 25 jun. 1996. Proceedings of the National Academy of Sciences. Disponível em: http://dx.doi.org/10.1073/pnas.93.13.6770.

XIE, Q.; CHO, H. J.; CALAYCAY, J.; MUMFORD, R. A.; SWIDEREK, K. M.; LEE, T. D. *et al.* Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. **Science**, [s.I.], v. 256, n. 5054, p.225-228, 10 abr. 1992. American Association for the Advancement of Science (AAAS). Disponível em: http://dx.doi.org/10.1126/science.1373522.

YANG, X. O.; PAPPU, B. P.; NURIEVA, R.; AKIMZHANOV, A.; KANG, H. S.; CHUNG, Y. *et al.* T Helper 17 Lineage Differentiation Is Programmed by Orphan Nuclear Receptors RORα and RORγ. **Immunity**, [s.I.], v. 28, n. 1, p.29-39, jan. 2008. Elsevier BV. Disponível em: http://dx.doi.org/10.1016/j.immuni.2007.11.016.

YAZICI, A. C.; TURSEN, U.; APA, D.D. *et al.* The changes in expression of ICAM-3, Ki-67, PCNA, and CD31 in psoriatic lesions before and after methotrexate treatment. **Archives of Dermatological Research**, [s.I.], v. 297, n. 6, p.249-255, 8 out. 2005. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1007/s00403-005-0602-8.

ZABA, L. C.; CARDINALE, I.; GILLEAUDEAU, P.; SULLIVAN-WHALEN, M.; SUÁREZ-FARIÑAS, M.; FUENTES-DUCULAN, J. *et al.* Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. **The Journal of Experimental Medicine**, [s.I.], v. 204, n. 13, p.3183-3194, 26 nov. 2007. Rockefeller University Press. Disponível em: http://dx.doi.org/10.1084/jem.20071094.

ZABA, L. C.; KRUEGER, J. G.; LOWES, M. A. Resident and "Inflammatory" Dendritic Cells in Human Skin. **Journal of Investigative Dermatology**, [s.I.], v. 129, n. 2, p.302-308, fev. 2009. Elsevier BV. Disponível em: http://dx.doi.org/10.1038/jid.2008.225.

ZHENG, Y.; DANILENKO, D.M.; VALDEZ, P.; KASMAN, I.; EASTHAM-ANDERSON, J.; WU, J. *et al.* Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. **Nature**, [s.I.], v. 445, n. 7128, p.648-651, fev. 2007. Springer Science and Business Media LLC. Disponível em: http://dx.doi.org/10.1038/nature05505.

APENDICES

ANEXO 1 - PARECER CONSUBSTANCIADO DO COMITE DE ÉTICA EM PESQUISA (CEP)

UNIVERSIDADE DE UBERABA - UNIUBE

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do perfil imunológico em pacientes com psoríase em placas em tratamento ou não com imunobiológicos

Pesquisador: DENISE BERTULUCCI ROCHA RODRIGUES

Área Temática:

Versão: 3

CAAE: 63049316.1.0000.5145

Instituição Proponente: SOCIEDADE EDUCACIONAL UBERABENSE

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.906.241

Apresentação do Projeto:

Trata-se da terceira apresentação da proposta "Avaliação do perfil imunológico em pacientes com psoríase em placas em tratamento ou não com imunobiológicos", da pesquisadora DENISE BERTULUCCI ROCHA RODRIGUES. A proposta foi colocada "em pendência" na reunião de 15/12/2016 com solicitação de adequar o TCLE e confirmar a confecção de um biobanco.

O projeto será realizado em parceria entre a UNIUBE e a UFTM e visa avaliar a presença de citocinas inflamatórias em paciente com psoríase. Os participantes serão abordados no ambulatório de psoríase- UFTM. Foi originalmente descrito pela proponente como um estudo "longitudinal, prospectivo, analítico, controlado, de prevalência e Multicêntrico".

A pesquisadora relata que "Serão avaliados 50 indivíduos divididos em 3 grupos. Um grupo de 20 pacientes com diagnóstico de psoríase em uso de imunobiológico, 1 grupo de 20 pacientes com psoríase em placas sem uso de imunobiológicos e um grupo de 10 indivíduos sadios. Será realizado culturas celulares do sangue periférico e após a citometria de fluxo do soro desses pacientes para comparar diferenças antes e após a medicação. Será realizado também extração de DNA/PCR, ELISA e/ou CBA, análise imunohistoquímica e imunofluorescência de amostras de tecido cutâneo afetados e não afetados"

Endereço: Av.Nene Sabino, 1801
Bairro: Universitário **CEP:** 38.055-500
UF: MG **Município:** UBERABA
Telefone: (34)3319-8811 **Fax:** (34)3314-8910 **E-mail:** cep@uniube.br

Objetivo da Pesquisa:

Retira-se da proposta:

Objetivo Primário

• Avaliar a presença de citocinas inflamatórias em pacientes com psoríase

Objetivo Secundário:

• Avaliar in situ a densidade de mastócito (Quinase e Triptase) em biopsias de pacientes com psoríase.• Avaliar citocinas in situ do perfil Th1, Th22,
Treg, Th-17 em pacientes com psoríase.• Avaliar os níveis séricos das citocinas do perfil Th1, Th22, Treg, Th-17 em pacientes com psoríase.•
Avaliar os níveis séricos e o perfil patogênico e não patogênico do perfil Th17.• Avaliar microbiota.

Avaliação dos Riscos e Benefícios:

Como relatado no parecer anterior, a pesquisa não expõe os participantes a riscos inerentes apenas à pesquisa, pois todos os procedimentos que serão realizados fazem parte do tratamento/avaliação do paciente. Retira-se da proposta: " Irão participar 50 indivíduos, sendo, 20 pacientes com diagnostico de psoríase em uso de imunobiológico, 20 pacientes com diagnostico de psoríase sem uso de imunobiológico e 10 indivíduos sadios que não apresentam psoríase com indicação de qualquer procedimento cirúrgico de reparação estética, cuja incisão será realizada em região submetida a tratamento cirúrgico (grupo controle)."

Sobre os riscos e benefícios, retira-se da proposta:

Riscos:

Os riscos para a execução do projeto são mínimos, pois será coletado material biológico dos pacientes já com indicação cirúrgica. As informações fornecidas, não permitirão qualquer acesso aos dados de identificação dos pacientes, já que os códigos de registro (códigos de caso) serão constituídos apenas por algarismos.

Benefícios:

Os benefícios da execução desta pesquisa residem na possibilidade de contribuir na elucidação das diferentes dúvidas que ainda existem quanto aos fatores imunológicos envolvidos no processo de evolução da psoríase e como os agentes imunobiológicos interferem no desenvolvimento da doença.

Entende-se assim que o paciente está preservado quanto a sua identidade, não sendo exposto pela participação. Os benefícios, se não diretos ao paciente, poderão favorecer futuros portadores

da doença.

Comentários e Considerações sobre a Pesquisa:

A pesquisa é pertinente e está bem detalhada.

Considerações sobre os Termos de apresentação obrigatória:

Todos os documentos pertinentes são anexados. TCLE, autorizações, e documentos pertinentes.

Recomendações:

A dúvida a respeito da criação do biobanco foi esclarecida e o TCLE apresentado encontra-se adequado.
Não há recomendações.

Conclusões ou Pendências e Lista de Inadequações:

conclui-se pela aprovação da proposta

Considerações Finais a critério do CEP:

O CEP-UNIUBE lembra ao coordenador do projeto o seu compromisso com o que dita a Resolução 466/2012, especialmente no que diz respeito à entrega dos relatórios parciais e final do projeto, ao CEP- UNIUBE. Além disso, solicita especial atenção quanto aos procedimentos de criação e manutenção do biobanco, de acordo com as normas da CONEP.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BASICAS_DO_PROJETO_840334.pdf	22/12/2016 17:55:33		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEPsoriase.docx	22/12/2016 17:55:04	DENISE BERTULUCCI ROCHA RODRIGUES	Aceito
Projeto Detalhado / Brochura Investigador	Projetopsoriaseatualizadodez16.pdf	21/12/2016 08:42:41	DENISE BERTULUCCI ROCHA	Aceito
Declaração de Instituição e Infraestrutura	AUTORIZACAOUFTM.pdf	14/12/2016 17:32:13	Rayane Bernardes Estevam	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	DECLARACAO.pdf	14/12/2016 09:23:03	Rayane Bernardes Estevam	Aceito
Outros	CARTAENCAMINHAMENTO.jpg	14/12/2016	Rayane Bernardes	Aceito

Outros	CARTAENCAMINHAMENTO.jpg	08:40:47	Estevam	Aceito
Cronograma	CRONOGRAMA1.pdf	14/12/2016 08:37:19	Rayane Bernardes Estevam	Aceito
Orçamento	ORCAMENTOFINANCEIRO.pdf	14/12/2016 08:30:03	Rayane Bernardes Estevam	Aceito
Folha de Rosto	FOLHADEROSTO.pdf	14/12/2016 08:09:39	Rayane Bernardes Estevam	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

UBERABA, 03 de Fevereiro de 2017

Assinado por:
Geraldo Thedei Junior
(Coordenador)

ANEXO 2 – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nome do paciente/sujeito da pesquisa

_____

Identificação (RG) do paciente/sujeito da pesquisa _____

Nome do responsável (quando aplicável):

_____

Identificação (RG) do responsável: _____

Título do projeto: Avaliação do perfil imunológico em pacientes com psoríase em placas em tratamento ou não com imunobiológicos.

Instituição onde será realizado: Universidade de Uberaba e Universidade Federal do Triângulo Mineiro

Pesquisador Responsável: Denise Bertulucci Rocha Rodrigues

Contato: (34) 991589234

CEP-UNIUBE: Av. Nenê Sabino, 1801 – Bairro: Universitário – CEP: 38055-500-Uberaba/MG, tel: 34-3319-8959 e-mail: cep@uniube.br

Você (ou Seu/Sua _____

_____ (colocar o nome e grau de parentesco do paciente/sujeito, no caso de menores) está sendo convidado para participar do projeto intitulado: Avaliação do perfil imunológico em pacientes com psoríase em placas em tratamento ou não com imunobiológicos de responsabilidade de Denise Bertuluci Rocha Rodrigues, desenvolvido na Universidade de Uberaba e Universidade Federal do Triângulo Mineiro.

Este estudo tem como objetivo avaliar a presença de citocinas e a densidade de mastócito em pacientes com psoríase.

A psoríase é uma das doenças dermatológicas mais relevantes na prática clínica devido à ausência de uma cura definitiva e ao impacto negativo que provoca na qualidade de vida dos doentes. Considerando que o diagnóstico da psoríase é essencialmente clínico, é de extrema importância que os profissionais de saúde tenham conhecimento aprofundado acerca desta patologia e das suas manifestações clínicas, de forma a estarem aptos a identificá-la num doente auxiliando-o na remissão dos sintomas e melhoria da sua qualidade de vida. Apesar de não haver atualmente uma cura definitiva para a psoríase, existe um conjunto de terapêuticas eficazes no controle da doença, dependendo este da adesão à terapêutica por parte dos doentes. Os recentes avanços no conhecimento da imunopatogênese da psoríase, em associação à toxicidade provocada pelas terapêuticas clássicas, conduziram à pesquisa e desenvolvimento de novas terapêuticas. É neste contexto que surgem os agentes imubiológicos no tratamento da psoríase, especificamente direcionados para as alterações imunopatogênicas da doença. A intervenção seletiva no sistema imune desta classe de agentes de imunológicos, torna-os uma abordagem terapêutica importante no controle de doenças auto-imunes como na psoríase.

Caso você participe, será necessário coleta de sangue na sua admissão e coleta de uma biopsia a ser realizada pelo médico. Você poderá ter algum desconforto quando receber uma picada para coletar o sangue do seu braço. Em relação à coleta do fragmento, será

feita durante o procedimento cirúrgico do paciente sob anestesia, já com indicação de coleta da biopsia, por isso, o procedimento realizado para pesquisa não acrescenta nenhum risco ao paciente. Espera-se que os benefícios decorrentes da sua participação nesta pesquisa sejam: contribuição para elucidação das diferentes dúvidas que ainda existem quanto aos fatores imunológicos envolvidos no processo de evolução da psoríase e como interferem no processo da doença.

Caso, aceite participar do estudo, o material humano, será coletado e armazenado ao longo da execução do projeto de pesquisa especifico de acordo com as normas técnicas, éticas e operacionais pré defenidas, seguindo a Resolução CNS Nº 441 de 12 de maio de 2011.

Os seus dados serão mantidos em sigilo e serão utilizados apenas com fins científicos, tais como apresentações em congressos e publicação de artigos científicos. Seu nome ou qualquer identificação sua (voz, foto, etc) jamais aparecerá.

Pela sua participação no estudo, você não receberá nenhum pagamento, e também não terá nenhum custo. Você pode parar de participar a qualquer momento, sem nenhum tipo de prejuízo para você ou para seu tratamento/atendimento *(OBS do CEP: caso trate-se de paciente/sujeito sob tratamento ou atendimento de qualquer tipo)*. Sinta-se à vontade para solicitar, a qualquer momento, os esclarecimentos que você julgar necessários. Caso decida-se por não participar, ou por não ser submetido a algum procedimento que lhe for solicitado, nenhuma penalidade será imposta a você, nem seu tratamento ou atendimento será alterado ou prejudicado *(OBS do CEP: caso trate-se de paciente/sujeito sob tratamento ou atendimento de qualquer tipo)*.

Você receberá uma cópia desse termo, assinada pela equipe, onde consta a identificação (nome e número de registro – se houver-) e os telefones da equipe de pesquisadores, caso você queira entrar em contato com eles.

_____

Nome do paciente (ou sujeito) ou responsável e assinatura

_____

Denise Bertulucci Rocha Rodrigues
(34) 991589234 - denise.rodrigues@uniube.br

_____

Rayane Bernardes Estevam
(34) 999600699 - rayanestevam@gmail.com

_____

Sandro Cecílio Furiati

(34) 998059979 – drsandrofuriati@gmail.com

ANEXO 3 - ARTIGO 1 “Th1, Th17, and Treg Responses are Diferently Modulated by TNF-α Inhibitors and Methotrexate in Psoriasis Patients”

Link para acesso: https://www.nature.com/articles/s41598-019-43899-9

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Th1, Th17, and Treg Responses are Differently Modulated by TNF-α Inhibitors and Methotrexate in Psoriasis Patients

Received: 27 November 2018
Accepted: 18 April 2019
Published online: 17 May 2019

Sandro C. Furiati [1], Jonatas S. Catarino [1,2], Marcos V. Silva [1], Rafaela F. Silva [1], Rayane B. Estevam [2], Reginaldo B. Teodoro [2], Sanivia L. Pereira [1,2], Meire Ataide [2], Virmondes Rodrigues Jr. [2] & Denise B. R. Rodrigues [1,2]

Psoriasis is a chronic, recurrent, immune-mediated, hyperproliferative inflammatory skin disease. The role of the adaptive immune system, particularly of Th1 and Th17 lymphocytes, has been regarded as prominent in the immunopathogenesis of psoriasis, as well as decreased Tregs function. Immunobiological drugs were administered in therapeutic pulses and a few studies evaluate their effects on the immune repertoire. The aim of this study was to evaluate the adaptive immune profile of patients with severe psoriasis under immunobiological treatment in two time points. Thirty-two psoriasis patients and 10 control patients were evaluated. In the group of psoriasis patients, 10 patients were on anti-TNF and 14 patients on methotrexate treatment, while 8 individuals were not treated. IL-17, IFN-γ, TNF-α, IL-6, IL-2, and IL-10 were analyzed. CD4 T cell intracellular cytokines were analyzed. It was observed that stimulation could significantly increase the production of IL-17, IFN-γ, TNF-α, and IL-10 only before anti-TNF pulse therapy. The activation of Th1 and Treg cells after stimulation was significantly higher before anti-TNF pulse. Patients on methotrexate or anti-TNF therapy produced significantly lower levels of TNF-α, IL-10, and IL-6. Furthermore, these patients showed a significant decrease in the activated CD4+ T cells. The treatment with immunomodulator or methotrexate modulates the activation of CD4+ T cells, and anti-TNF treatment appears to have a modulating effect on the activation and production of Th1, Th17, and Treg cells.

Psoriasis is a chronic inflammatory skin disease that affects about 2–3% of the world's population[1–3]. It is caused by a combination of genetic and environmental factors, as well as by complex interactions between the innate and adaptive immune system[4]. There are several types of psoriasis, and the clinical variant called psoriasis vulgaris or plaque psoriasis is the most common, affecting 85–90% of the patients[5]. Up to one third of these patients may be associated with the articular manifestation of the disease, called arthropathic psoriasis[6]. Clinically, psoriasis vulgaris is usually characterized by lesions on well-demarcated round to oval erythematous plaques, with thick, dry, silvery adherent scales[7]. These scales are the result of a hyperproliferative epidermis in which keratinocytes mature early, leading to an incomplete cornification with the retention of keratinocyte nuclei in the stratum corneum (parakeratosis). This results in epidermal hyperplasia (acanthosis) and elongation of papillary ridges[8]. Histologically, these lesions appear as an inflammatory infiltrate, consisting mainly of dendritic cells (DCs), macrophages, and T cells in the dermis, as well as neutrophils and some T cells in the epidermis. The erythematous characteristic of the lesions is due to the increase in the number of tortuous capillaries that reach the skin surface through papillary ridges[9].

IFN-α and other cytokines produced by innate immune system cells, such as interleukin (IL)-1, IL-6, tumor necrosis factor alpha (TNF-α), and IFN-γ, stimulate the activation and maturation of myeloid DCs, which are considered the key cells that link the innate immune system and the adaptive immune system[10]. In addition to

[1]University of Uberaba, Uberaba, Minas Gerais, Brazil. [2]Laboratory of Immunology, Department of Biological Sciences, and Cefores, Triângulo Mineiro Federal University, Uberaba, Minas Gerais, Brazil. Sandro C. Furiati and Jonatas S. Catarino contributed equally. Correspondence and requests for materials should be addressed to D.B.R.R. (email: denise.rodrigues@uniube.br)

being activated by these cytokines, myeloid DCs also have TLRs (Toll-like receptors), through which they can directly interact with the auto-RNA–LL-37 complex, causing their concomitant activation, thus starting to produce several proinflammatory cytokines (IL-23, TNF-α, IL-12, IL-6, IL-20, and IL-1)[11].

The mature myeloid DCs migrate to the lymph nodes, where, with the help of cytokines they produce, they induce the differentiation of naïve $CD4^+$ T cells (Th0) into Th17, Th1, or Th22, thus triggering the activity of the adaptive immune system in the inflammatory process of psoriasis[12]. These differentiated $CD4^+$ T cells migrate to the dermis, where, by interacting with the innate immune system cells, they produce their typical inflammatory cytokines[13]. Th17 cells produce IL-17, IL-6, TNF-α, IL-21, and IL-22[14]; Th1 cells produce TNF-α and IFN-γ[15]; and Th22 cells produce IL-22 and TNF-α[16]. The inflammatory process is initially dominated by Th17 cells, but in stable psoriasis plaques, it subsequently shifts towards a process dominated by IFN-γ-producing Th1 cells[17,18].

Keratinocytes are the main targets of these cytokines, especially of IL-17, IFN-γ, TNF-α, and IL-22, which directly or indirectly lead to their hyperproliferation, as well as induce them to produce various other cytokines, chemokines, and antimicrobial peptide, which in turn continue to stimulate the activation and recruitment of cells of the innate and adaptive immune system to the lesions, thus perpetuating the inflammatory process of psoriasis[9]. IL-17 and IL-23 are considered the key cytokines in the immunopathogenesis of psoriasis, and they are referred to as the IL-23–IL-17 axis[19]. In the immunopathogenesis of psoriasis, TNF-α promotes the activation and maturation of DCs, which, in turn, produce more IL-23[20].

Regulatory T cells (Tregs), identified as $CD4^+$ $CD25^+$ $Foxp3^+$ cells, play a crucial role in immune tissue homeostasis and in self-tolerance because they are capable of suppressing effector cell activation and proliferation through the production of IL-10, hence controlling inflammatory processes in the body[21]. However, in psoriasis, this regulatory capacity is diminished or absent[22]. Studies indicate a malfunction of Tregs or a decrease in their numbers in psoriatic lesions[23]. T regulatory cells expressing mLAP (membrane latency-associated peptide) is a population of human Tregs that is different from classic CD4+Foxp3+CD25 high natural Tregs[24]. LAP is a propeptide that favors the release of TGF-β into the extracellular milieu and for this reason is related to several functions in T regulatory cells such as suppressive activity, survival and peripheral conversion of iTregs and Th17[25]. T regulatory cells expressing mLAP shows a potent immunosuppressive activity and are augmented in some neoplasic disorders, such as lung adenocarcinoma [26]and Colorectal Cancer[27].

Systemic treatment of psoriasis is reserved for moderate and severe cases, and it consists in the use of medications that can inhibit or reduce the immune response, thus reducing inflammation and inflammatory cell infiltration, as well as improving psoriatic lesions[28]. Methotrexate and anti-TNF are the major immunosuppressive medications used in the systemic treatment of psoriasis[29]. Methotrexate is a folic acid analogue and antimetabolite that has an effect on both circulating and cutaneous lymphocytes[30]. *In vitro*, keratinocytes are more resistant to its cytotoxic effects than T cells, thus proving its immunosuppressive properties[31]. It is considered to be antineoplastic, antipsoriatic, and antirheumatic [32], and it inhibits neutrophil chemotaxis and the release of TNF-α, IFN-γ, IL-12, and IL-6, ultimately leading to its anti-inflammatory activity[33]. Anti-TNF is used for treating psoriasis in case of therapeutic failure or contraindication of methotrexate . Immunobiologicals (IBs) can be used alone or, in some cases, concomitantly with methotrexate, and there are five immunobiologicals currently approved for the treatment of psoriasis in Brazil, three of which are anti-TNF drugs (adalimumab, infliximab, and etanercept) and two interleukin inhibitors (ustekinumab and secukinumab). Ustekinumab is also an inhibitor of IL-23, and secukinumab is also an inhibitor of IL-17A[34].

Anti-TNF agents block the soluble fraction of TNF-α and the transmembrane fraction of TNF that is expressed on the membrane of cells producing this cytokine[35]. TNF-α blockade decreases the activation of myeloid DCs, which are important sources of IL-23 and play a vital role in the differentiation of naïve $CD4^+$ T cells into Th17 cells[28]. After TNF-α blockade, the activation of these cells is also decreased, and so is the differentiation of Th0 into Th1 or Th22 and the production of their respective standard cytokines[36]. A study of 21 patients using anti-TNF adalimumab in China showed a decrease in the circulating populations of Th17, Th1, and Th22 cells[37]. In addition to these effects, the number of $CD4^+$ $CD25^+$ $Foxp3^+$ cells increased in the peripheral blood of psoriasis patients after treatment with biologicals. This increase was associated with a good clinical response and a reduction in the psoriasis area severity index (PASI), although some patients had decreased numbers of Tregs after the biological treatment, which was associated with worsening of the disease.

However, the effect of TNF-blocker therapy at different intervals of the therapeutic cycle has not yet been clarified . This knowledge is necessary to understand the reasons for the low therapeutic response and the short duration of its effects in some individuals. Therefore, the aim of the present study was to evaluate the pattern of the adaptive immune response in patients with stable severe psoriasis without treatment and patients undergoing systemic therapy with methotrexate or anti-TNF agents at different intervals of the therapeutic cycle.

Materials and Methods

Patients . Thirty-two patients with stable severe psoriasis undergoing treatment at the dermatology clinic, who agreed to participate in this study, were recruited . The diagnosis was confirmed by biopsy. Ten patients had previous assessment of the PASI, with a result above 10, and were regularly using anti-TNF immunobiological medication (adalimumab) ; 14 patients had previous diagnosis of severe psoriasis (PASI > 10) and had been on methotrexate treatment for at least 2 months; 8 patients had previous diagnosis of severe psoriasis (PASI > 10) and had not been on therapy for at least 2 months. Patients' mean age and gender distribution are presented at Table 1. Ten healthy control subjects were selected paired by gender. The study was approved by the Ethics Committee (*CAAE*) of UNIUBE, under the protocol number 63049316l.100005145and was conducted at the University of Uberaba and at the Clinical Hospital (*Hospitaldas Clínicas*) of the Federal University of Triângulo Mineiro (UFTM), Uberaba, Minas Gerais, Brazil. The patients were subjected to blood collection for cell culture, with the aim of analyzing the production of cytokines in the culture supernatant and the T cell phenotype by flow cytometry . The patients who used IBs were submitted to two blood samplings; one immediately before

	Gender (Male/Female)	Age – mean (Male/Female)
Healthy subjects	5/5	41.1/42.8
Patients without therapy	6/2	44.6/56.5
Patients treated with methotrexate	7/7	52.8/46.6
Patients treated with adalimumab	5/5	43.2/46.1

Table 1. Gender and age mean of patients and healthy subjects.

receiving the therapeutic dose (day 0) and the other in the middle of the cycle (day 7), between applications. All the methods were performed in accordance with both institutional guidelines and all individuals who accepted to participate in this study signed an informed consent form after clarification.

Isolation and culture of peripheral blood mononuclear cells. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation, using Histopaque-1077 (Sigma-Aldrich, St Louis, Mo, USA), for 30 mins at 400 × g, at 24 °C. The cells were then resuspended in RPMI 1640 medium (Sigma-Aldrich) containing 50 mM HEPES buffer (Gibco, Grand Island, NY, USA), 10% of inactivated fetal bovine serum (Gibco), 2 mM of L-glutamine (Gibco), and 40 mg/mL of gentamicin (Neoquímica, Anápolis, Brazil) to a final concentration of 2×10^6 cells/mL. PBMCs were cultured in 24-well microplates (Falcon, San Jose, CA, USA) in the presence or absence of anti-CD3 and anti-CD28 for 48 h at 37 °C in a 5% CO_2 atmosphere. Cells (for immunophenotyping) and supernatants (for cytokine measurement) were collected after 48 h. The cells were collected after 48 h for immunophenotyping, and the supernatants were collected after 48 h and stored at −70 °C for the measurement of soluble mediators using the cytometric bead array (CBA) (BD Bioscience, San Diego, CA, USA).

Flow cytometry for analysis of the expression of surface molecules, cytokines, and transcription factors. For the analysis of the expression of surface molecules, cytokines, and transcription factors in cells derived from the 48 h culture, cells were washed for 10 min at 400 g, at 4C, in Hanks' medium supplemented with 10% human AB^+ serum, previously submitted to inactivation of the complement system, and incubated for 30 mins. The cells were split in three tubes and then labeled with antibodies targeting surface molecules: tube 1 - anti-CD4–PE Cy5 and anti-CD69–PE, tube 2 - anti-CD4–PE Cy5, anti-CD25–FITC, and anti-h/m-LAP–APC (BD Pharmingen) or tube 3 - respective control isotypes antibodies, and maintained at 4C for 30 mins. After that, the cells were washed three times for 10 min at 400 × g and 4 °C to remove excess antibodies, resuspended in 500 µL PBS containing 0.5% paraformaldehyde, and stored at 4C in a dark chamber until flow cytometry analysis. For intracellular detection, the cells were fixed and permeabilized with 250 µL of Cytofix/Cytoperm (BD Biosciences) at 4C for 30 mins. Next, they were washed three times in Perm/Wash (BD Biosciences), containing 10% fetal bovine serum (Sigma-Aldrich). In tube 1 were added anti-FoxP3–PE, in tube 2 anti-IL-17–Alexa Fluor 488, and anti-IFN-γ–Alexa Fluor 647 and in tube 3 respective intracellular isotype control antibodies. The cells were incubated at 4C for 30 min. At the end of this period, the cells were washed in Perm/Wash three more times for 10 mins at 400 g, at 4 °C, resuspended in 200 µL of 0.5% paraformaldehyde and stored in a dark chamber at 4C until flow cytometry analysis. Two tubes were placed parallel to each labeled sample: A tube without antibodies and a tube containing control isotopes compatible with the fluorescence used. Data acquisition (50,000 events/tube) was performed using a FACSCalibur cytometer (BD Biosciences), using the CellQuest software (BD Biosciences). Data analysis was performed using FlowJo 10.0.6 software (Tree Star) by isolating leukocyte populations through gates established according to the size (FSC) and granularity (SSC) characteristics of T cell populations.

Cytokine concentrations in the culture supernatants. Production of IL-17A, IFN-γ, TNF-α, IL-10, IL-6, and IL-2 was analyzed simultaneously in the culture supernatants of PBMCs, using the CBA Human Inflammatory Cytokine Kit (BD Biosciences), according to the manufacturer's instructions. The samples and recombinant cytokines were incubated with microspheres of different fluorescence intensities conjugated with captured antibodies specific for each cytokine. Then, PE-conjugated antibodies specific for each cytokine were added. After incubation, the microspheres were washed with the corresponding solutions and analyzed on a FACSCalibur cytometer (BD Biosciences) using the CellQuest software (BD Biosciences). The microspheres specific for each cytokine were separated due to the fact that they emitted different intensities of fluorescence at 660 nm, and the amount of cytokines conjugated with each of them was separated by fluorescence intensity at 585 nm. Sample data and data on recombinant cytokines were collected and subsequently analyzed using FCAP Array 2.0 software (Soft Flow, Pécs, Hungary), and cytokine concentrations were determined using standard curves.

Statistical analysis. Statistical analysis was performed using the GraphPad Prism software (version 6.00; GraphPad Software, La Jolla, CA, USA). The Wilcoxon Signed Rank Test was used to compare two continuous variables in the same patients. The Kruskal-Wallis test was used to compare three or more groups, followed by Dunn's post-hoc test. The difference was considered significant when $p < 0.05$.

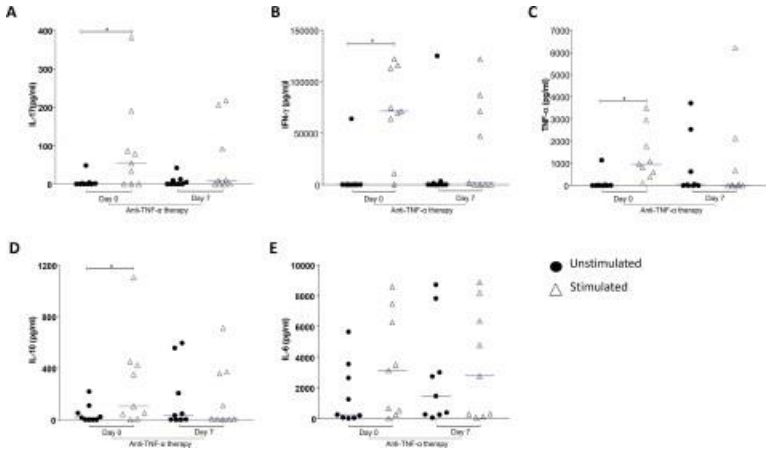


Figure 1. Analysis of cytokines in culture supernatant of PBMC, incubated for 48 h in the presence of medium (unstimulated) or with anti-CD3 and anti-CD28 mAbs (stimulated). The PBMCs were obtained prior to pulse therapy (day 0) and during pulse therapy (day 7) of patients with severe plaque psoriasis treated with anti-TNF agents. (**A**) IL-17 levels (*Wilcoxon; $p < 0.05$), (**B**) IFN-γ levels (*Wilcoxon; $p < 0.05$), (**C**) TNF-α levels (Wilcoxon; $p > 0.05$), (**D**) IL-10 levels (*Wilcoxon; $p < 0.05$), (**E**) IL-6 levels (*Wilcoxon; $p < 0.05$). The results are expressed in pg/mL. *Indicates statistical significance.

Results

Treatment with anti-TNF downregulates the production of IL-17A, IFN-γ, TNF-α, and IL-10. Cytokine analyses of psoriatic patients on anti-TNF therapy were performed on two occasions: prior to pulse therapy (day 0) and 7 days after the anti-TNF therapy (day 7). IL-17, IFN-γ, TNF-α, IL-10, IL-6, and IL-2 levels were analyzed by CBA of the PBMC culture supernatant 48 h after stimulation with anti-CD3 and anti-CD28 or after no stimulation (Fig. 1). Analysis of IL-17 levels in the PBMC supernatants showed that anti-CD3 and anti-CD28 stimulation significantly increased IL-17 production just before the pulse therapy (day 0), compared to the untreated supernatants ($p = 0.031$) (Fig. 1A). On the other hand, there was no significant increase in IL-17 production after anti-CD3 and anti-CD28 stimulation of the supernatants of patients whose pulse therapy was in progress (day 7) (Fig. 1A). Similarly, anti-CD3 and anti-CD28 stimulation significantly increased IFN-γ production only before pulse therapy (day 0), when compared with the untreated supernatants (day 0) ($p = 0.007$) (Fig. 1B). There was no significant increase in IFN-γ production after anti-CD3 and anti-CD28 stimulation of the supernatants of patients whose pulse therapy was in progress (day 7) (Fig. 1B). Similar to IFN-γ, anti-CD3 and anti-CD28 stimulation could significantly increase TNF-α production only before pulse therapy (day 0), compared with the untreated supernatants (day 0) ($p = 0.007$) (Fig. 1C). However, when TNF-α levels were observed under anti-CD3 and anti-CD28 stimulation, there was no significant difference between the levels in the supernatants of patients whose pulse therapy was in progress (Fig. 1C). When evaluating IL-10 levels, anti-CD3 and anti-CD28 stimulation significantly increased IL-10 production in the supernatants of patients who had not undergone pulse therapy (day 0), when compared to the untreated supernatants (day 0) ($p = 0.0078$) (Fig. 1D). There was no significant increase in IL-10 production after anti-CD3 and anti-CD28 stimulation of the supernatants of patients whose pulse therapy was in progress (day 7) (Fig. 1D). No significant upregulation was observed in the levels of IL-6 (Fig. 1E).

Treatment with anti-TNF downregulates T cell activation. The response of T cells after anti-CD3 and anti-CD28 stimulation and CD4 T cells were analyzed by the expression of the cell activation marker CD69 and intracellular cytokines. T cells obtained from PBMCs before pulse therapy (day 0) and 7 days after the anti-TNF therapy (day 7) were analyzed by flow cytometry after 48 h of culture. As for cell activation markers, the expression of CD69 in $CD4^+$ T cells stimulated with anti-CD3 and anti-CD28 was significantly higher in patients before initiating pulse therapy (day 0) than in the same patients, but without cell stimulation ($p = 0.02$) (Fig. 2G). There was no significant difference between IL-17-producing $CD4^+$ cells ($IL\text{-}17^+IFN\text{-}\gamma^-$) that were or were not stimulated with anti-CD3 and anti-CD28 (Fig. 2H). Nonetheless, when comparing IFN-γ-producing $CD4^+$ cells ($IL\text{-}17^-IFN\text{-}\gamma^+$) stimulated with anti-CD3 and anti-CD28 before pulse therapy (day 0), there was a significant increase in the percentage of IFN-γ-producing cells compared with the non-stimulated cells (day 0) ($p < 0.013$)

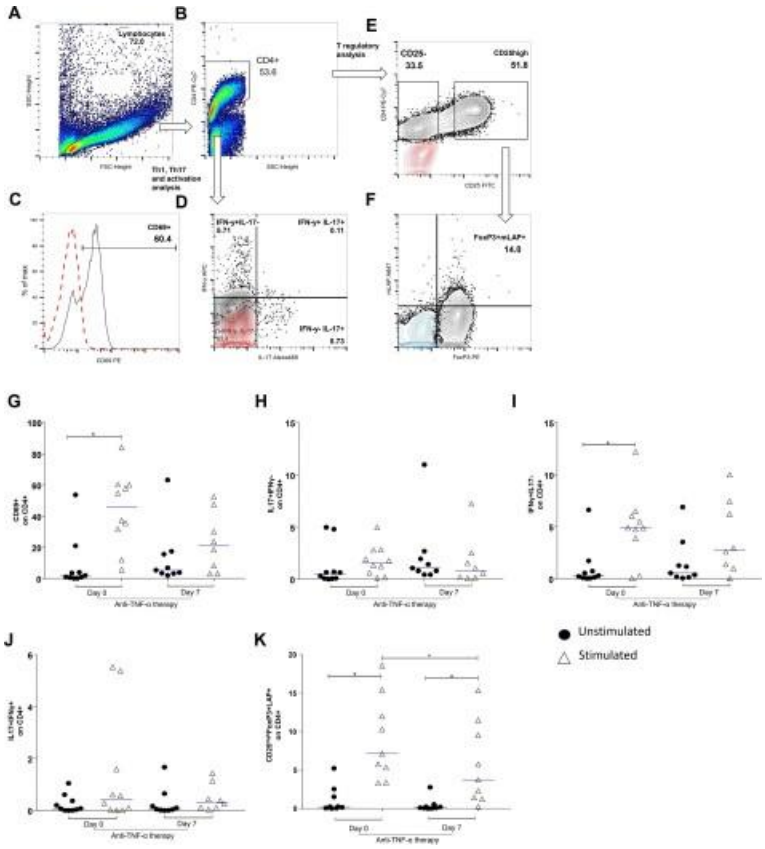


Figure 2. Flow cytometric analysis of cell activation markers and intra-cellular cytokine expression on CD4+ T-lymphocytes (TL): PBMCs were obtained prior to pulse therapy (day 0) and during pulse therapy (day 7) of patients with severe plaque psoriasis treated with anti-TNF agents. PBMC, cultured for 48 h in the presence of medium (unstimulated) or with anti-CD3 and anti-CD28 mAbs (stimulated). After recovered, PBMC were incubate with appropriate mAbs and isotype controls. Panels A to F show gates strategies for analysis. In panels C–E the red dots indicate the labeling of the control isotypes. In panel F, the blue dots indicate the $CD25^-$ population. Panel G: $CD69^+$ on CD4+ TL (Wilcoxon $p < 0.05$), panel H: $IL\text{-}17^+IFN\text{-}\gamma^-$ on CD4+ LT (Wilcoxon $p > 0.05$), panel I: $IL\text{-}17^-$ $IFN\text{-}\gamma^+$ on CD4+ LT (Wilcoxon $p < 0.05$), panel J: $IL\text{-}17^+IFN\text{-}\gamma^+$ on CD4+ LT (Wilcoxon $p > 0.05$) and panel K: $CD25^{high}FoxP3^+LAP^+$ on CD4+ LT (Wilcoxon $p < 0.05$). The results are expressed in percentage on the CD4+ TL. *Indicate statistical significance.

(Fig. 2I). On the other hand, there was no significant difference when comparing $CD4^+IFN\text{-}\gamma^+$ cells and IL-17-producing cells ($IL\text{-}17^+$ $IFN\text{-}\gamma^+$) with or without stimulation (Fig. 2J). CD4 T lymphocytes $CD25^{hi}FoxP3^+$ LAP^+ showed a significant increase in the percentage of Treg cells after stimulation with anti-CD3 and anti-CD28 before (day 0) and after (day 7) pulse therapy (day 0) compared with the percentage of cells in the same patients, but without stimulation ($p = 0.003$). Furthermore, before pulse therapy (day 0), this response was significantly

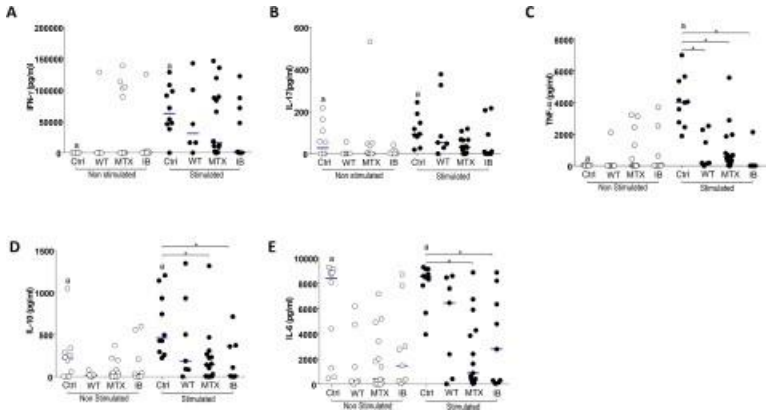


Figure 3. Analysis of cytokines in culture supernatant of PBMC, incubated for 48h in the presence of medium (unstimulated) or with anti-CD3 and anti-CD28 mAbs (stimulated). The PBMCs were obtained from healthy subjects (Ctrl), psoriasis patients without therapy (WT), patients treated with methotrexate (MTX), and patients treated with immunobiologicals (IB). Panel A: IFN-γ levels (^{a}Wilcoxon; $p < 0.05$), panel B: IL-17 levels (^{a}Wilcoxon $p < 0.05$), panel C: TNF-α levels (^{a}Wilcoxon; $p < 0.05$ and *Kruskal-Wallis $p < 0,05$), panel D: IL-10 levels (^{a}Wilcoxon $p < 0.05$ and *Kruskal-Wallis $p < 0,05$), panel E: IL-6 levels (^{a}Wilcoxon; $p = 0.05$ and *Kruskal-Wallis $p < 0,05$). The results are expressed in pg/mL. a and *indicate statistical significance.

higher than at after (day7) ($p = 0.004$) (Fig. 2K). Figure 2A–F illustrate the acquisition strategies. In Fig. 2C–E, the red dots indicate the labeling of the control isotypes. In Fig. 2F, the blue dots indicate the $CD25^-$ population.

Treatment with anti-TNF or methotrexate downregulates the production of cytokines in the culture supernatants . Psoriasis patients were grouped as follows: patients without any therapy, patients treated with methotrexate, and patients treated with anti-TNF. The effects of anti-TNF therapy were analyzed 7 days after initiation of the therapy. There were no significant differences in IFN-γ and IL-17 levels in the supernatants between the different patient groups. However, only the control group showed significant upregulation of IFN-γ and IL-17 when stimulated, compared with the unstimulated cultures (Fig. 3A,B). TNF-α production after anti-CD3 and anti-CD28 stimulation was significantly lower in untreated psoriatic patients, in patients treated with methotrexate, and in patients treated with anti-TNF, when compared to healthy control individuals ($p = 0.017$, $p = 0.004$, and $p = 0.003$, respectively). Furthermore, when stimulated, only the control group presented significant upregulation of IFN-γ and IL-17 compared with the unstimulated cultures (Fig. 3C).

It was observed that, after anti-CD3 and anti-CD28 stimulation, IL-10 levels were significantly lower in psoriasis patients treated with methotrexate and in patients on anti-TNF treatment than in healthy control subjects ($p = 0.029$ and $p = 0.030$, respectively) (Fig. 3D). Again, only in the control group was the anti-CD3 and anti-CD28 stimulation able to induce a significant production of IL-10 (Fig. 3D).

Similarly, IL-6 levels were significantly lower in patients treated with methotrexate or treated with anti-TNF in comparison to healthy controls ($p = 0.002$ and $p = 0.033$, respectively). Here, patients without any therapy showed a significant upregulation of IL-6 after anti-CD3 and anti-CD28 stimulation ($p = 0.001$) (Fig. 3E).

Treatment with anti-TNF and methotrexate modulates CD_4^+ T cell activation . In patients grouped according to treatment, the response of T cells after anti-CD3 and anti-CD28 stimulation was analyzed by the expression of the cell activation markers, CD69 and CD4, and intracellular cytokines. It was observed that the expression of CD69 in $CD4^+$ T cells after anti-CD3 and anti-CD28 stimulation was significantly lower in patients on methotrexate and in patients receiving anti-TNF than in healthy control subjects ($p = 0.009$ and $p = 0.02$, respectively). Only in the control group was the anti-CD3 and anti-CD28 treatment able to significantly upregulate the number of activated $CD4^+$ T cells ($p = 0.002$) (Fig. 4A).

There was no significant difference in the percentages of IL-17-producing helper T cells ($IL\text{-}17^+IFN\text{-}\gamma^-$), IFN-γ-producing cells ($IL\text{-}17^-IFN\text{-}\gamma^+$), or $IFN\text{-}\gamma^+IL\text{-}17^+$ double-positive $CD4^+$ cells among the different groups of patients. However, in control subjects, anti-CD3 and anti-CD28 stimulation was able to significantly upregulate the number of $IFN\text{-}\gamma^+$, $IL\text{-}17^+$, and $IFN\text{-}\gamma^+IL\text{-}17^+$ double positive $CD4^+$ T cells ($p = 0.01$, $p = 0.01$, and $p = 0.01$, respectively) (Fig. 4B–D). On the contrary, it was observed that, after stimulation with anti-CD3 and anti-CD28,

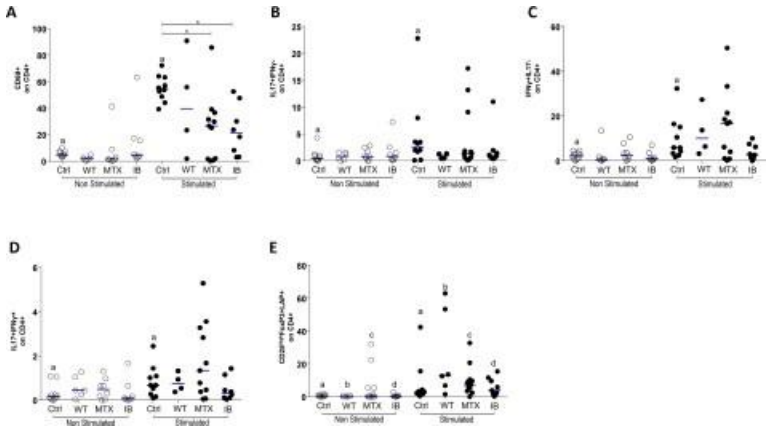


Figure 4. Flow cytometric analysis of cell activation markers and intra-cellular cytokine expression on CD4 + T-lymphocytes (TL): The PBMCs were obtained from healthy subjects (Ctrl), psoriasis patients without therapy (WT), patients treated with methotrexate (MTX), and patients treated with immunobiologicals (IB). PBMC, incubated for 48h in the presence of medium (unstimulated) or with anti-CD3 and anti-CD28 mAbs (stimulated). After recovered, PBMC were incubate with appropriate mAbs and isotype controls. Panel A: $CD69^+$ on CD4 + TL (^{a}Wilcoxon; $p = 0.05$ and *Kruskal-Wallis $p < 0,05$), panel B: $IL\text{-}17^+IFN\text{-}\gamma^-$ on CD4 + LT (^{a}Wilcoxon $p > 0.05$), panel C: $IL\text{-}17^-$ IFN-γ^+ on CD4 + LT (^{a}Wilcoxon $p < 0.05$), panel D: $IL\text{-}17^+IFN\text{-}\gamma^+$ on CD4 + LT (^{a}Wilcoxon $p > 0.05$) and panel E: CD25+ $FoxP3^+LAP^+$ on CD4 + LT (^{a}Wilcoxon; p 0.05 and *Kruskal-Wallis $p < 0,05$). The results are expressed in percentage on the CD4 + TL. A and *indicates statistical significance.

there was a significant increase in CD4 + T cells of a regulatory phenotype ($CD25^{hi}FoxP3^+LAP^+$) in untreated psoriasis patients compared with the group treated with methotrexate ($p = 0.03$) (Fig. 4F).

Discussion

Psoriasis is an immune -mediated skin disease with various clinical subtypes of different pathogenesis; the patients enrolled in this study had stable plaque psoriasis. The major advances in the treatment of severe psoriasis were made with the introduction of biological products. However, only long-term results of clinical trials and post-marketing surveillance can demonstrate the success of these new therapies [38]. Understanding the mechanisms of the actions of these products is of great importance for choosing the appropriate therapy and so are the suspension criteria or changes in the course of the treatment . The complex interactions of innate and adaptive immune system cells, in conjunction with the molecules released during the immune response, may trigger the modulation of elements other than those that are targeted during a therapy. Thus, several cytokines have been studied as blocking targets in biological therapies.

IL-17A is considered the key cytokine in the inflammatory process of psoriasis because it has a direct influence on the activation and hyperproliferation of keratinocytes [39,40]. There is a decrease in the expression of this cytokine *in situ* when biological therapies are used to treat moderate to severe psoriasis and, although Th17 cell products are modulated rapidly during the course of treatment with biological therapy, Th1 and Th2 cell products are modulated at a later phase, months after the disease has significantly improved [28]. Similarly, IL-12 and IL-23 inhibitors prevent Th1 and Th17 cells to secret their cytokines, thus decreasing the production of IFN-γ and IL-17A, respectively [40].

In this study, we analyzed 32 patients with psoriasis; ten of them under treatment with TNF inhibitors, receiving one dose each 15 days. All patients, on the day preceding the therapeutic pulse (day 0), presented more psoriasis symptoms than on day 7, after receiving TNF inhibitors. These clinical signs are important and indicative of the need to administer another dose and the effectiveness of the treatment .

In the present study, it was observed that the stimulation of cells from patients with anti-CD3 and anti-CD28 led to a heterogeneous response, which may be related to the genetic diversity of human population. Although heterogeneous, this response led to a significant increase in IL-17A levels only in day 0, on which the assays were performed just before anti-TNF pulse therapy. On the contrary, when stimulating these cells with anti-CD3 and anti-CD28, no significant increase in IL-17A levels 7 days after anti-TNF therapy was observed. Investigating the number of $IL\text{-}17^+CD4^+$ T lymphocytes, we observed that anti-CD3 and anti-CD28 stimulation did not induce

a significant increase in the number of IL-17-producing cells in both samples, suggesting that the TNF blockade consistently inhibited the generation of Th17 cells.

Our results are in agreement with the literature, which shows that the use of anti-TNF and other cytokine inhibitors such as anti-IL-12/anti-IL-23 and anti-IL-17A directly or indirectly reduces the production of IL-17A[28]. Hence, it is suggested that anti-TNF therapy can inhibit IL-17A production.

IFN-γ is another cytokine involved in the pathogenesis of psoriasis, and it plays an important role in the inflammatory process of the disease, as it seems to be associated with the perpetuation of the inflammatory process by having a synergistic effect with IL-17A in the activation of keratinocytes[41]. Studies have shown that increased serum levels of IFN-γ are directly associated with active disease[42]. The use of biological therapies has also been shown to be effective in reducing IFN-γ levels, as they reduce the activity of TNF-α-producing Th1 cells, and therefore, in reducing IFN-γ levels[43]. In our study, stimulation with anti-CD3 and anti-CD28 was able to significantly increase IFN-γ levels only in day 0, in which the assays were performed just before the pulse therapy with anti-TNF. On the contrary, stimulation with anti-CD3 and anti-CD28 7 days after anti-TNF therapy was not able to significantly increase IFN-γ levels. Investigating the number of IFN-γ$^+$CD4$^+$ T lymphocytes, we observed that anti-CD3 and anti-CD28 stimulation induced a significant increase in the number of IFN-γ-producing cells only in day 0, suggesting that IFN-γ-producing cells are more resistant to the TNF blockage.

Like IL-17 and IFN-γ, TNF-α is a crucial cytokine in the inflammatory process of psoriasis, and it is produced by cells of both the innate and adaptive immune systems. Circulating levels of TNF-α, IFN-γ, and IL-17A are directly correlated with the severity of psoriasis[44]. Biological therapy has been shown to reduce TNF-α levels in most studies. Anti-TNF-α medications block the free soluble fraction and membrane fraction of this cytokine[45]. In this study, anti-CD3 and anti-CD28 stimulation significantly increased TNF-α production only in day 0, on which the assays were performed just before anti-TNF pulse therapy. These data are consistent with the literature that has demonstrated that immunosuppressive medications such as methotrexate and anti-TNF can reduce the levels of this interleukin during treatment [46].

In addition to the participation of proinflammatory cytokines involved in psoriasis, the role of anti-inflammatory cytokines has also been investigated. IL-10 plays a critical role in protecting against tissue damage in acute inflammatory processes[47], thus suppressing the expression of inflammatory cytokines produced by effector cells[48]. Studies indicate that psoriasis patients are deficient in IL-10[49]. Some authors suggest that there is a mild deficiency in the expression of IL-10 messenger RNA in psoriasis in comparison with other inflammatory skin diseases[50]. In this study, under anti-CD3 and anti-CD28 stimulation, IL-10 was also found to increase significantly just before anti-TNF pulse therapy, hence suggesting that the regulatory immune mechanism was able to be elicited in these patients, even though it was not sufficient to block the proinflammatory cytokines that were produced. Therefore, these data suggest that the use of anti-TNF interferes with the immune response, negatively modulating the production of IL-17, IFN-γ, TNF-α, and IL-10 after *in vitro* stimulation with anti-CD3 and anti-CD28. This modulatory property was also demonstrated in an *in vitro* model, where adding anti-TNF-α antibodies to PBMC cultures downregulated the production of IL-17, IFN-γ, TNF-α, and IL-10[51].

Interestingly, the different TNF inhibitors appear to have distinct mechanisms of action. Anti-TNF has been shown to act more quickly on Th17 than on Th1 populations, and the inhibition of the Th1 population seems to have a stronger correlation with clinical improvement [28].

The analysis of regulatory T cells (CD25^{hi}FoxP3$^+$LAP$^+$) after *in vitro* activation of CD4$^+$ T cells with anti-CD3 and anti-CD28 demonstrated that anti-TNF therapy modulates the number of cells of the Treg phenotype, indicating that the number of Treg cells is reduced during the pulse therapy. These results suggest that the ability to expand Treg cells *in vitro* is modulated by anti-TNF pulse therapy; however, it appears to be restored by the end of the therapeutic treatment. Although some studies have shown that there is a decrease in the number and function of Tregs in psoriasis[21], other studies have shown an increase in the number of CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells in peripheral blood *ex vivo*[52,53]. This is the first study evaluating LAP + Treg cells in psoriasis. The evaluation of the time course of treatment with anti-TNF showed inhibition of the activation of CD4$^+$ T cells. Moreover, that the down modulation of IFN-γ -producing cells are highly dependent of anti-TNF therapy, while IL-17 –producing cells remain stable down regulated at the 2 time point of treatment analyzed. Due T regulatory-derived TGF-β acts in immunoregulatory mechanisms and Th17 differentiation [54], the possible link between LAP expression in T cells and suppressive versus IL-17 induction activity needs to be further characterized in psoriasis.

In this study, we also evaluated patients on methotrexate and anti-TNF therapy. It was observed that, regardless of the treatment approach, TNF-α levels were significantly lower in relation to the control group after *in vitro* stimulation. The role of immunobiological agents in the reduction of TNF-α by is well documented[28,35,54]. Methotrexate is an immunosuppressive folic acid antagonist that is able to inhibit the activation of lymphocytes and macrophages, thus modulating cytokines and inhibiting neutrophil chemotaxis[55]. Studies have also shown that methotrexate is able to block TNF-α production by T cells and macrophages [56]. In this study, low levels of TNF-α were observed even in untreated patients. It is important to emphasize that this group comprised patients that had a stable chronic psoriasis profile despite not undergoing therapy for at least 30 days. In addition, it must be noted that some of these patients had previously underwent methotrexate treatment, which was unsuccessful. Furthermore, it is important to note that all patients who received TNF blockers were previously treated with methotrexate and had poor therapeutic response. These results suggest that both TNF blockers and methotrexate are capable of modulating TNF-α synthesis. Furthermore, patients with stable psoriatic disease retain the ability to modulate TNF-α production. IL-6 is also a proinflammatory cytokine, and it plays a key role in the differentiation of Th17 cells and in the activation of myeloid DCs[57]. Studies have shown the correlation between the levels of this cytokine and disease activity, as well as its regulation in immunosuppressive therapies[58]. Our study showed that treatments both with methotrexate and immunobiologicals significantly reduced the stimulated production of IL-6.

Interestingly, in our results, patients on methotrexate or anti-TNF therapy produced less IL-10 than the control group. These data suggest that these therapeutic strategies simultaneously affect the production of pro-inflammatory cytokines and anti-inflammatory cytokines such as IL-10. Thus, our results suggest that the beneficial effects of the treatment are not associated with a higher production of IL-10 or development of Treg cells, but rather with the direct effects of both methotrexate and immunobiologicals on the blockage of other inflammatory cytokines, such as TNF-α, IL-17, and IFN-γ.

In this study, the expression of cell activation markers and intracellular cytokines in $CD4^+$ T cells in untreated and treated patients was compared with the control group. It was observed that patients on methotrexate or anti-TNF therapy had a lower number of activated $CD4^+$ T cells after anti-CD3 and anti-CD28 stimulation than the control group. Evaluation of intracellular cytokines in these cells showed no differences between IFN-γ^+, IL-17^+, or IL-17^+IFN-γ^+ double-positive populations. Our results indicate that both the treatment with methotrexate and with TNF inhibitors negatively modulates $CD4^+$ T cell activation, but does not interfere with their ability to produce IL-17 or IFN-γ. The increase in the number of cells with a Treg phenotype in untreated patients may indicate a functional failure of these cells, at least in patients with stable psoriatic disease.

Conclusions

Treatment with IBs and methotrexate modulates the activation of $CD4^+$ T cells. Anti-TNF appears to have a modulatory effect on the activation and production of cytokines by Th1, Th17, and Treg lymphocytes in a distinct manner. Down modulation of IFN-γ-producing cells are highly dependent on anti-TNF therapy, while IL-17-producing cells remain stable down regulated at the 2 time point of treatment analyzed.

References

1. Michalek, I. M., Loring, B. & John, S. M. A systematic review of worldwide epidemiology of psoriasis. *J Eur Acad Dermatol Venereol* **31**, 205–212, https://doi.org/10.1111/jdv.13854 (2017).
2. Parisi, R. *et al.* Global epidemiology of psoriasis: a systematic review of incidence and prevalence. *The Journal of investigative dermatology* **133**, 377–385, https://doi.org/10.1038/jid.2012.339 (2013).
3. Lima, X. T., Minnillo, R., Spencer, J. M. & Kimball, A. B. Psoriasis prevalence among the 2009 AAD National Melanoma/SkinCancer Screening Program participants. *J Eur Acad Dermatol Venereol* **27**, 680–685, https://doi.org/10.1111/j.1468-3083.2012.04531.x (2013).
4. Grine, L., Dejager, L., Libert, C. & Vandenbroucke, R. E. An inflammatory triangle in psoriasis: TNF, type I IFNs and IL-17. *Cytokine & growth factor reviews* **26**, 25–33, https://doi.org/10.1016/j.cytogfr.2014.10.009 (2015).
5. Griffiths, C. E. M. & Barker, J. Pathogenesis and clinical features of psoriasis. *The Lancet* **370**, 263–271, https://doi.org/10.1016/S0140-6736(07)61128-3 (2007).
6. Ranza, R. *et al.* Prevalence of Psoriatic Arthritis in a Large Cohort of Brazilian Patients with Psoriasis. *The Journal of Rheumatology* **42**, 829–834, https://doi.org/10.3899/jrheum.140474 (2015).
7. Lowes, M. A. *et al.* Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *The Journal of investigative dermatology* **128**, 1207–1211, https://doi.org/10.1038/sj.jid.5701213 (2008).
8. Jiaravuthisan, M. M., Sasseville, D., Vender, R. B., Murphy, F. & Muhn, C. Y. Psoriasis of the nail: Anatomy, pathology, clinical presentation, and a review of the literature on therapy. *Journal of the American Academy of Dermatology* **57**, 1–27, https://doi.org/10.1016/j.jaad.2005.07.073 (2007).
9. Nestle, F. O., Di Meglio, P., Qin, J.-Z. Z. & Nickoloff, B. J. Skin immune sentinels in health and disease. *Nature reviews Immunology* **9**, 679–691, https://doi.org/10.1038/nri2622 (2009).
10. Nestle, F. O. *et al.* Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *The Journal of experimental medicine* **202**, 135–143, https://doi.org/10.1084/jem.20050500 (2005).
11. Ganguly, D. *et al.* Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. *The Journal of experimental medicine* **206**, 1983–1994, https://doi.org/10.1084/jem.20090480 (2009).
12. Khasawneh, A. *et al.* Myeloid but not plasmacytoid blood DCs possess Th1 polarizing and Th1/Th17 recruiting capacity in psoriasis. *Immunol Lett* **189**, 109–113, https://doi.org/10.1016/j.imlet.2017.04.003 (2017).
13. Cai, Y., Fleming, C. & Yan, J. New insights of T cell sin the pathogenesis of psoriasis. *Cellular & Molecular Immunology* **9**, https://doi.org/10.1038/cmi.2012.15 (2012).
14. Korn, T., Bettelli, E., Oukka, M. & Kuchroo, V. K. IL-17 and Th17 Cells. *Immunology* **27**, 485–517, https://doi.org/10.1146/annurev.immunol.021908.132710 (2009).
15. Austin, L. M., Ozawa, M., Kikuchi, T., Walters, J. B. & Krueger, J. G. The majority of epidermal T cell sin Psoriasis vulgaris lesions can produce type 1 cytokines, interferon-gamma, interleukin-2, and tumor necrosis factor-alpha, defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cell sin psoriatic patients. *J Invest Dermatol* **113**, 752–759, https://doi.org/10.1046/j.1523-1747.1999.00749.x (1999).
16. Boniface, K. *et al.* A role for T cell-derived interleukin 22 in psoriatic skin inflammation. *Clinical & Experimental Immunology* **150**, 407–415, https://doi.org/10.1111/j.1365-2249.2007.03511.x (2007).
17. Christophers, E., Metzler, G. & Röcken, M. Bimodal immune activation in psoriasis. *British Journal of Dermatology* **170**, 59–65, https://doi.org/10.1111/bjd.12631 (2014).
18. Chiricozzi, A., Romanelli, P., Volpe, E., Borsellino, G. & Romanelli, M. Scanning the Immunopathogenesis of Psoriasis. *International Journal of Molecular Sciences* **19**, 179, https://doi.org/10.3390/ijms19010179 (2018).
19. Gooderham, M. & Papp, K. Selective Phosphodiesterase Inhibitors for Psoriasis: Focus on Apremilast. *BioDrugs* **29**, 327–339, https://doi.org/10.1007/s40259-015-0144-3 (2015).
20. Mudigonda, P. *et al.* Interleukin-23 and interleukin-17: importance in pathogenesis and therapy of psoriasis. *Dermatology online journal* **18**, 1 (2012).
21. Mattozzi, C. *et al.* Importance of regulatory T cell sin the pathogenesis of psoriasis: review of the literature. *Dermatology (Basel, Switzerland)* **227**, 134–145, https://doi.org/10.1159/000353398 (2013).
22. Yang, L. *et al.* Impaired function of regulatory T cell sin patients with psoriasis is mediated by phosphorylation of STAT3. *Journal of dermatological science* **81**, 85–92, https://doi.org/10.1016/j.jdermsci.2015.11.007 (2016).
23. Soler, D. C. & McCormick, T. S. The Dark Side of Regulatory T Cells in Psoriasis. *Journal of Investigative Dermatology* **131**, 1785–1786, https://doi.org/10.1038/jid.2011.200 (2011).
24. Gandhi, R. *et al.* Cutting edge: human latency-associated peptide+ T cells a novel regulatory T cell subset. *Journal of immunology* **184**, 4620–4624, https://doi.org/10.4049/jimmunol.0903329 (2010).
25. Tran, D. Q. TGF-beta: the sword, the wand, and the shield of FOXP3(+) regulatory T cells. *Journal of molecular cell biology* **4**, 29–37, https://doi.org/10.1093/jmcb/mjr033 (2012).

26. Islas-Vazquez, L. *et al.* LAP TGF-Beta Subset of CD4(+)CD25(+)CD127(−) Treg Cells is Increased and Overexpresses LAP TGF-Beta in Lung Adenocarcinoma Patients. *BioMed research international* **2015**, 430943, https://doi.org/10.1155/2015/430943 (2015).
27. Mahalingam, J. *et al.* CD4(+) T cells expressing latency-associated peptide and Foxp3 are an activated subgroup of regulatory T cells enriched in patients with colorectal cancer. *PLoS One* **9**, e108554, https://doi.org/10.1371/journal.pone.0108554 (2014).
28. Zaba, L. C. *et al.* Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *The Journal of experimental medicine* **204**, 3183–3194, https://doi.org/10.1084/jem.20071094 (2007).
29. Mahil, S. K., Capon, F. & Barker, J. N. Update on psoriasis immunopathogenesis and targeted immunotherapy. *Seminars in immunopathology* **38**, 11–27, https://doi.org/10.1007/s00281-015-0539-8 (2016).
30. Cronstein, B. N. Molecular therapeutics. Methotrexate and its mechanism of action. *Arthritis and rheumatism* **39**, 1951–1960 (1996).
31. Elango, T., Thirupathi, A., Subramanian, S., Dayalan, H. & Gnanaraj, P. Methotrexate normalized keratinocyte activation cycle by overturning abnormal keratins as well as deregulated inflammatory mediators in psoriatic patients. *Clinica Chimica Acta* **451**, 329–337, https://doi.org/10.1016/j.cca.2015.10.020 (2015).
32. Sbidian, E. *et al.* Factors associated with the choice of the first biologic in psoriasis: real-life analysis from the Psobioteq cohort. *Journal of the European Academy of Dermatology and Venereology* **31**, 2046–2054, https://doi.org/10.1111/jdv.14406 (2017).
33. Bressan, A. L., Silva, R. S., Fontenelle, E. & Gripp, A. C. Immunosuppressive agents in Dermatology. *Anais brasileiros de dermatologia* **85**, 9–22 (2010).
34. Kim, J. *et al.* Molecular Phenotyping Small (Asian) versus Large (Western) Plaque Psoriasis Shows Common Activation of IL-17 Pathway Genes but Different Regulatory Gene Sets. *The Journal of investigative dermatology* **136**, 161–172, https://doi.org/10.1038/JID.2015.378 (2016).
35. Horiuchi, T., Mitoma, H., Harashima, S.-I., Tsukamoto, H. & Shimoda, T. Transmembrane TNF-α: structure, function and interaction with anti-TNF agents. *Rheumatology* **49**, 1215–1228, https://doi.org/10.1093/rheumatology/keq031 (2010).
36. Aravena, O. *et al.* Anti-TNF therapy in patients with rheumatoid arthritis decreases Th1 and Th17 cell populations and expands IFN-γ-producing NK cell and regulatory T cell subsets. *Immunobiology* **216**, 1256–1263, https://doi.org/10.1016/j.imbio.2011.07.006 (2011).
37. Luan, L., Han, S., Wang, H. & Liu, X. Down-regulation of the Th1, Th17, and Th22 pathways due to anti-TNF-α treatment in psoriasis. *International Immunopharmacology* **29**, 278–284, https://doi.org/10.1016/j.intimp.2015.11.005 (2015).
38. Rønholt, K. & Iversen, L. Old and New Biological Therapies for Psoriasis. *International Journal of Molecular Sciences* **18**, 2297, https://doi.org/10.3390/ijms18112297 (2017).
39. Lynde, C. W., Poulin, Y., Vender, R., Bourcier, M. & Khalil, S. Interleukin 17A: Toward a new understanding of psoriasis pathogenesis. *Journal of the American Academy of Dermatology* **71**, 141–150, https://doi.org/10.1016/j.jaad.2013.12.036 (2014).
40. Campa, M., Mansouri, B., Warren, R. & Menter, A. A Review of Biologic Therapies Targeting IL-23 and IL-17 for Use in Moderate-to-Severe Plaque Psoriasis. *Dermatology and therapy* **6**, 1–12, https://doi.org/10.1007/s13555-015-0092-3 (2016).
41. Kryczek, I. *et al.* Induction of IL-17+ T cell trafficking and development by IFN-gamma: mechanism and pathological relevance in psoriasis. *Journal of immunology (Baltimore, Md.: 1950)* **181**, 4733–4741 (2008).
42. Arican, O., Aral, M., Sasmaz, S. & Ciragil, P. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators of inflammation* **2005**, 273–279, https://doi.org/10.1155/MI.2005.273 (2005).
43. Nograles, K. E. & Krueger, J. G. Anti-cytokine therapies for psoriasis. *Experimental cell research* **317**, 1293–1300, https://doi.org/10.1016/j.yexcr.2011.01.024 (2011).
44. Jacob, S. E., Nassiri, M., Kerdel, F. A. & Vincek, V. Simultaneous measurement of multiple Th1 and Th2 serum cytokines in psoriasis and correlation with disease severity. *Mediators of inflammation* **12**, 309–313, https://doi.org/10.1080/09629350310001619753 (2003).
45. Lucka, T. C. *et al.* Efficacy of systemic therapies for moderate-to-severe psoriasis: a systematic review and meta-analysis of long-term treatment. *Journal of the European Academy of Dermatology and Venereology: JEADV* **26**, 1331–1344, https://doi.org/10.1111/j.1468-3083.2012.04492.x (2012).
46. Gottlieb, A. B. *et al.* TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. *Journal of immunology (Baltimore, Md.: 1950)* **175**, 2721–2729 (2005).
47. Rojas, J. M., Avia, M., Martín, V. & Sevilla, N. IL-10: A Multifunctional Cytokine in Viral Infections. *Journal of Immunology Research* **2017**, 1–14, https://doi.org/10.1155/2017/6104054 (2017).
48. Ouyang, W., Rutz, S., Crellin, N. K., Valdez, P. A. & Hymowitz, S. G. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annual review of immunology* **29**, 71–109, https://doi.org/10.1146/annurev-immunol-031210-101312 (2011).
49. Al-Robaee, A. A., Al-Zolibani, A. A., Al-Shobili, H. A., Kazamel, A. & Settin, A. IL-10 implications in psoriasis. *International journal of health sciences* **2**, 53–58 (2008).
50. Cheng, J. *et al.* A study on the expression of interleukin (IL)-10 and IL-12 P35, P40 mRNA in the psoriatic lesions. *Journal of Tongji Medical University = Tong ji yi ke da xue xue bao* **21**, 86–88 (2001).
51. da Silva, D. A. *et al.* TNF-α blockade impairs *in vitro* tuberculous granuloma formation and down modulate Th1, Th17 and Treg cytokines. *PLOS One* **13**, https://doi.org/10.1371/journal.pone.0194430 (2018).
52. Richetta, A. G. *et al.* CD4+ CD25+ T-regulatory cells in psoriasis. Correlation between their numbers and biologics-induced clinical improvement. *European journal of dermatology: EJD* **21**, 344–348, https://doi.org/10.1684/ejd.2011.1362 (2011).
53. Zhang, L., Yang, X.-Q. Q., Cheng, J., Hui, R.-S. S. & Gao, T.-W. W. Increased Th17 cells are accompanied by FoxP3(+) Treg cell accumulation and correlated with psoriasis disease severity. *Clinical immunology (Orlando, Fla.)* **135**, 108–117, https://doi.org/10.1016/j.clim.2009.11.008 (2010).
54. Hop, H. T. *et al.* Activation of NF-kB-Mediated TNF-Induced Antimicrobial Immunity Is Required for the Efficient Brucella abortus Clearance in RAW 264.7 Cells. Frontiers in Cellular and Infection. *Microbiology* **7**, 437, https://doi.org/10.3389/fcimb.2017.00437 (2017).
55. Seitz, M. *et al.* Methotrexate action in rheumatoid arthritis: stimulation of cytokine inhibitor and inhibition of chemokine production by peripheral blood mononuclear cells. *British journal of rheumatology* **34**, 602–609 (1995).
56. Neurath, M. F. *et al.* Methotrexate specifically modulates cytokine production by T cells and macrophages in murine collagen-induced arthritis (CIA): a mechanism for methotrexate-mediated immunosuppression. *Clinical and experimental immunology* **115**, 42–55 (1999).
58. Park, G., Lee, S., Han, J.-Y. & Oh, D.-S. Altered TNF-α response by Aconibal® and methotrexate in a lipopolysaccharide-induced setting of inflammatory conditions: Potential on a synergistic combination. *Journal of Ethnopharmacology* **213**, 191–197, https://doi.org/10.1016/j.jep.2017.11.020 (2018).
57. Hanley, T. L. & Yiu, Z. Z. Role of IL-17 in plaque psoriasis: therapeutic potential of ixekizumab. *Therapeutics and clinical risk management* **13**, 315–323, https://doi.org/10.2147/TCRM.S111107 (2017).

Acknowledgements

This work was funded by CNPq, FAPEMIG, CAPES, FUNEPU and CEFORES. The funders had no role in the study design, data collection and analysis, the decision to publish, or preparation of the paper.

Author Contributions

V.R.J., D.B.R.R., Study design, conceptualization, project administration, manuscript revision, data analysis. J.S.C., M.V.S., Data collection, data managed, investigation, manuscript writing. S.C.F., R.F.S., R.B.E., M.S.A., R.B.T., S.L.P., Patients assistance, investigation and patients recruitment .

Additional Information

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

ANEXO 4 - ARTIGO 2 “Molecules that modulate inflammatory responses are expressed in affected and non-affected skin from patients with plaque psoriasis: a case-control study”

Molecules that modulate inflammatory responses are expressed in affected and non-affected skin from patients with plaque psoriasis: a case-control study

Rayane Bernardes Estevam[1], Sandro Cecílio Furiati[1], Chamberttan Souza Desidério[1], Jonatas da Silva Catarino[1], Meire Ataíde[1], Betânia Maria Ribeiro[1], Natalia Sene[2], Laís Milagres Barbosa[1], Virmondes Rodrigues Junior[1,3*], Denise Bertulucci Rocha Rodrigues[1,2].

[1]Federal University of Triângulo Mineiro (UFTM), Uberaba, MG, Brazil.

[2]Laboratory of Biopathology and Molecular Biology, University of Uberaba (UNIUBE), Uberaba, MG, Brazil.

[3]INCT- Neuroimunomodulação

Corresponding author:

*Virmondes Rodrigues Junior

Universidade Federal do Triângulo Mineiro

Av. Vigário Carlos, 1801, Bairro Abadia CEP 38.055-500, Uberaba MG, Brazil

Telephone number: +55 34 3319 8815

Fax number: +55 34 3314 8910

E-mail address: virmondes.rodrigues@uftm.edu.br

ABSTRACT: Psoriasis is of the disease is characterized by an interaction between innate and adaptive immune systems mediated by the inappropriate activation of T cells, associated with a complex network of cells and inflammatory mediators. T Biopsies were collected one directly from the affected tissue and another from the non-affected tissue. Methotrexate therapy was used in 15 psoriasis patients, 6 were not in treatment. The material was processed for mRNA extraction, cDNA synthesis and, finally, for qPCR of IL-17, IL-23, IL-22, IL-10, IFN-γ, TNF-α, TGF-βR2, ARG1, ARG2, iNOS and GLUT1. The results showed that the in situ gene expression of all evaluated genes was higher in the affected and non-affected tissue samples from patients with psoriasis. Expression of IL-17, IFN-γ, ARG1 and ARG2 genes was higher in the affected tissue than in the non-affected tissue. Methotrexate therapy was associated with high mRNA expression of IFN-γ, IL-10, TGF-βR2 and ARG2 in patients with plaque psoriasis. The overexpression of these molecules in affected and non-affected tissues suggests that the disturbance of immune response psoriasis is beyond the clinically affected skin. In sucha affected skin areas, a strong response of Th1 and Th17 prototypes cytokines are observed, in association of Type 2 macrophages markers.

Keywords: Psoriasis; Cytokines; Arginase; GLUT; Methotrexate.

INTRODUCTION

Psoriasis is a chronic hyperproliferative inflammatory disease characterized by the appearance of well-demarked erythematous-squamous plaques (GRINE *et al.*, 2015). Usually, the lesions are pink/reddish, covered with dry, whitish scales that alternate in acute periods with phases of improvement and worsening (RYAN, 2008). The disease is characterized by the activation of the immune response mediated by persistent T cell stimulation, release pro-inflammatory cytokines causing cutaneous and articular manifestations (RIVAS BEJARANO; VALDECANTOS, 2013). It is known that the innate and adaptive immune systems are strongly associated with the development of psoriasis (SCHÖN; BOEHNCKE, 2005). The adaptive immune system, mediated mainly by Th1 and Th17 T cells, plays an important role in maintaining the inflammatory process in psoriasis (HARRINGTON *et al.*, 2005). Th17 cells are particularly abundant in psoriatic lesions as Th1 cells. Expanded activated T helper cells Th 1, Th17 and Th22 migrate to the skin where they start to release interferon (IFN-γ), tumor necrosis factor (TNF-α), IL-6, IL-22 and IL -23 (KIMURA; NAKA; KISHIMOTO, 2007; NOGRALES *et al.*, 2008). This inflammatory environment is associated with neovascularization, hyperproliferation, hyperplasia and keratinocyte activation, which release more inflammatory cytokines and chemokines (NESTLE *et al.*, 2009).

Several studies have pointed out the IL-17A as the major cytokine in psoriasis pathogenesis. It stimulates keratinocyte to product several chemokines, including CCL-20, which attracts more Th17 cells and dendritic cells, inducing the production of IL-23 (BREMBILLA; SENRA; BOEHNCKE, 2018; HOMEY *et al.*, 2000). IL-17A also acts in synergy with TNF-α to maintain ongoing inflammatory process (LYNDE *et al.*, 2014). The circulating levels of TNF-α, IFN-γ and IL-17A were described to be directly correlated with the severity of the disease (JACOB *et al.*, 2003). Hence, selective

inhibitors of TNF-α and IL-17 are used in the treatment of patients with psoriasis that are refractory to drugs such as methotrexate (PATEL *et al.*, 2013; ZABA *et al.*, 2007). This synergism also applies to IFN-γ, which stimulates the release of IL-1 and IL-23 by dendritic cells, maintaining the IL-23/Th17 axis (KRYCZEK *et al.*, 2008).

Studies have shown that TNF-α blockers inhibit the production of IL-17, IFN-γ, TNF-α and IL-10 by PBMCs of patients with psoriasis (FURIATI *et al.*, 2019). This pro-inflammatory environment is counterbalanced by anti-inflammatory cytokines, including IL-10 and TGF-β, which are cytokines produced by regulatory T cells (Treg). These cytokines have a strong modulating effect, especially on the Th1 and Th17 responses (MATTOZZI *et al.*, 2013). Serum levels of IL-10 are reduced in patients with active psoriasis. IL-10 inhibits the production of important pro-inflammatory cytokines and chemokines (ASADULLAH *et al.*, 1998).

The balance of this inflammatory environment may be indirectly evaluated in the macrophage profiles. Inflammatory macrophages (M1) have a high phagocytic capacity and a high production of reactive oxygen and nitrogen intermediates, whereas M2 macrophages are associated with tissue repair and proliferation processes (SICA; MANTOVANI, 2012). Nitric oxide synthase (NOS) has been associated with keratinocyte proliferation and differentiation (BRUCH-GERHARZ; RUZICKA; KOLB-BACHOFEN, 1998). On the other hand, arginase 1 (ARG1) and arginase 2 (ARG2) act on L-arginine competing with NOS to the same substrate (DIZIKES *et al.*, 1986).

Keratinocyte hyperproliferation and increased blood vessel formation implies high local energy consumption. Glucose is the main source of energy for cells, and glucose transporter 1 is the most common glucose transporter in humans (GLUT1). GLUT1 regulates basal glucose uptake in epidermal basal cells (GHERZI *et al.*, 1992). Even though the metabolic consequences of increased glucose transport are not yet

understood, the expression of GLUT1 appears to contributes to keratinocyte proliferation and angiogenesis. Studies have shown an increase in the expression of GLUT1 in psoriatic plaques (TOCHIO; TANAKA; NAKATA, 2013).

In order to contribute to the understanding of this complex scenario, analyses of mRNA expression of molecules associated with the inflammatory process (IL-17, 1L-23, IL-22, IFN-γ, TNF-α), molecules associated with regulation inflammation (IL-10 and TGF-βR2), and molecules associated with macrophage subtypes (ARG1, ARG2 and iNOS), and of an indicator of metabolic activity (GLUT1).

MATERIALS AND METHODS

Patients

The sample consisted of 26 patients, 21 of which were diagnosed with moderate to severe psoriasis (PASI > 10). All patients underwent biopsy performed with a 5mm punch; one section was collected from the affected tissue and another section was collected from a non-affected tissue. These sections were placed in RNAlater and frozen at -90º to perform the qPCR. 15 out of the 21 patients were being treated with methotrexate (MTX) for a period of three months or more, and 6 patients remained without medication. The control group consisted of biopsies from 5 healthy patients who had undergone breast plastic surgery.

The study was approved by the Ethics Committee (*CAAE*) of the University of Uberaba (*UNIUBE*) under protocol number 63049316.1.100005145. The investigation was performed at the School Hospital of the Federal University of Triângulo Mineiro (*UFTM*) in partnership with *UNIUBE*, in Uberaba, Brazil. All eligible patients were informed about the nature of the study and agreed to signed the Free and Informed Consent Form (FICF).

RNA Extraction

Tissue samples were obtained for one year. RNA extraction, reverse transcription and qPCR were performed at the same time to minimize the risk of bias in the procedures. Total RNA extraction was performed using an RNA extraction kit (RNA SV Total RNA Isolation System, (Promega, Madison, Wisconsin, USA), according to the manufacturer's recommendations. The tissue sections, which had been stored at -80ºC, were processed by mechanical maceration in a tube containing lysis buffer. Samples were heated at 70 ºC for 3 minutes and centrifuged at 13000xg at 4 ºC for 10 minutes. Supernatant was transferred to new tubes and 200 µL of 95% ethanol was added. The material was transferred to a separation silica tube and washed by centrifugation (13000xg at 4 ºC). The presence of contaminating DNA was eliminated by DNase treatment at room temperature for 15 minutes. The material was washed twice. RNA was obtained by adding 30 µL of nuclease-free water and centrifuged at 13000xg at 4 ºC for 1 minute and used for synthesis of complementary DNA (cDNA).

cDNA synthesis

The cDNA was synthesized from RNA,using an reverse transcriptase kit according to the manufacturer's recommendation (GoScrip Reverse Transcriptase, Promega). This material was placed in a PTC-100 thermal cycler (MJ Research, St. Bruno, Quebec, Canada) at 70 ºC for 5 minutes. After cooling, dNTP, reverse transcriptase and Reaction Buffer were added. This reaction was placed in the thermal cycler for another cycle of 1 hour at 42 ºC. cDNA samples were then frozen at -20 ºC until use.

Quantitative PCR reactions (qPCR)

The quantitative mRNA expressions of IL-17 (cod.Hs001743383), IL-23 (cod.Hs00900828), IL-22 (cod.Hs01574154), IL-10 (cod.Hs00961622), IFN-γ (cod.Hs00989291), TNF-α (cod.Hs00174128), TGF-βR2 (cod.Hs00234253), ARG1 (cod.Hs00163660), ARG2 (cod.Hs00982833), iNOS (cod.Hs01075529) and GLUT1 (cod.Hs00892681) were analyzed by real-time PCR of samples of cDNA section from patients diagnosed with psoriasis and from healthy control tissue. TaqMan primers and probes (Applied Biosystems, Waltham, Massachusetts, USA) were used in the StepOnePlus real-time PCR device (Applied Biosystems) with primers suitable for such reactions, and using β-actin (cod.Hs01060665). The results were analyzed based on the cycle threshold (CT) value, and the arithmetic formula used to achieve relative quantification was $\Delta\Delta Ct = \Delta$ Ct (treated) – Ct (control) (LIVAK; SCHMITTGEN, 2001).

Statistical analysis

The data were analyzed using the GraphPad Prism 8.2 software. Mann-Whitney, Kruskal-Wallis and Wilcoxon tests were used for data analysis. The results were considered statistically significant when $p < 0.05$.

RESULTS

All the patients diagnosed with psoriasis presented the active form of the disease, so their genes were found to have a significantly higher expression in the samples of affected and non-affected tissue when compared to the samples of control patients ($p < 0.05$).

The age of the psoriasis patients ranged from 23 to 69 years, with an mean of 49.05. A demografic profile of the experimental groups is presented in Table 1.

In this observational case-control study, we analyzed mRNA expression for cytokines that modulate the inflammatory response especially implicated in plaque

psoriasis and extended the analysis to other markers of macrophage activity and glucose metabolism. Furthermore, the analysis of the expression of these markers in the affected and unaffected skin regions showed that some of the markers are highly expressed even in normal-looking skin. We believe that the number of patients enrolled in this study, despite being modest, was sufficient to support the conclusions, since the *p*-values were highly significant in the statistical analysis.

Relative expression of IL-17 and IFN-γ genes is increased in tissues affected by psoriasis

Relative mRNA expressions of IFN-γ and IL-17 were found to be significantly higher in affected tissues than in non-affected tissues ($p = 0.03$ and 0.04). Moreover, the expression of the two genes was more pronounced in the skin of patients with psoriasis than in the skin of healthy control subjects ($p < 0.0001$) (FIGURES 1A and 1B).

Relative mRNA expressions of IL-23 ($p = 0.001$) (FIGURE 1C), IL-22 ($p= 0.01$) (FIGURE 1D), IL-10 ($p= 0.0058$) (FIGURE 1E), TNF-α ($p= 0.009$) (FIGURE 1F), and TGF-βR2 ($p= 0.0025$) (FIGURE 2A) were significantly higher in the skin of patients with psoriasis than in the skin of control subjects. Nonetheless, the expression of these molecules did not show any significant difference between the skin of affected and non-affected tissues from the same patient.

Tissues affected by psoriasis express higher amounts of TGF-βR2, ARG1 and ARG2 mRNA

Relative mRNA expressions of TGF-βR2, ARG1 and ARG2 were significantly higher in affected skin than in non-affected areas ($p< 0.0001$). Furthermore, TGF-βR2,

ARG1 and ARG2 expressions in biopsies of patients with psoriasis were significantly higher than in the skin of control subjects ($p < 0.0001$) (FIGURES 2A, 2B and 2C).

Tissues affected and non-affected by psoriasis express higher amounts of iNOS and GLUT1 mRNA than tissues of the control group

Relative mRNA expressions of iNOS and GLUT1 were significantly higher in the skin of psoriasis patients than in the skin of control subjects ($p = 0.007$ and $p = 0.001$). However, there was no significant difference between the expression of these genes in affected and non-affected skin biopsies from the same patient (FIGURES 2D and 2E).

Psoriasis patients on methotrexate therapy showed increased mRNA expression of IFN-γ, IL-10, TGF-βR2 and ARG2

When evaluating the relative mRNA expression of ARG2, IFN-γ, and IL-10 in patients who were using MTX and in patients without medication, a significant increase was observed in the non-affected tissues of patients being treated with MTX when compared to untreated patients ($p < 0.05$) (FIGURES 3A, 3B and 3C).

On the other hand, TGF-β-R2 expression was significantly higher in biopsies obtained from the affected skin of patients using methotrexate than in biopsies from non-affected skin ($p < 0.05$) (FIGURE 3D). The other cytokines analyzed did not show significant differences between groups.

DISCUSSION

This study showed a significant increase in mRNA expression of inflammatory mediator in affected and non-affected skin byopsies from patients with psoriasis when compared with skin byopsies from healthy subjects. Furthermore, in patients with psoriasis, a significant increase in mRNA expression of IL-17 and IFN-γ in skin biopsies of affected tissues were observed when compared with non-affected tissues.

IL-17 family members play a major role on inflammation, increasing neutrophil recruitment. Experimental murine models have demonstrated that the development of psoriatic inflammation depends on the increase in IL-17 expression (HAWKES *et al.*, 2018). In a previous study which also investigated mRNA expression of IL-17, biopsies of lesional skin showed higher levels of IL-17 expression in psoriatic lesions than in normal controls, thus linking IL-17 to the pathogenesis of psoriasis (JIAWEN; DONGSHENG; ZHIJIAN, 2004). As observed for IL-17, mRNA expression of IFN-γ was found to be increased in affected tissues in comparison with non-affected tissues. These cytokines have been linked to psoriasis lesions, both in tissue expression and in the secretion of PBMC. A synergistic effect of IFN-γ and IL-17 has been reported, leading to a sustained inflammatory condition and subsequent keratinocyte activation. In addition, both IL-17 and IFN-γ have been shown to be modulated downwards by successful therapy using anti-TNF-α antibodies in patients with psoriasis (FURIATI *et al.*, 2019). The greater expression of IL-17 and IFN-γ mRNA in affected areas of the skin, compared with non-affected areas, emphasizes their role in the pathogenesis of psoriasis lesions.

Paradoxically, when comparing affected and non-affected tissues, an increased expression of TGF-βR, ARG1 and ARG2 was observed in biopsies of the lesional area. In the same environment where inflammatory cytokines are observed at a high level, molecules associated with M2 macrophages and with cell regeneration and proliferation are also increased. When compared to the control subjects, all these mediators were

increased in patients with psoriasis, regardless of the biopsy area. However, when comparing affected and non-affected areas, they were significantly elevated in biopsies of the affected area. These findings characterize an active inflammatory process mediated by IL-17 and IFN-γ, with cell proliferation and the presence of M2 macrophage markers such as ARG1, ARG2 and TGF-βR, all existing at the same time (KEPKA-LENHART *et al.*, 2000; LI *et al.*, 2001).

The mRNA expression of other molecules associated with inflammation was increased when compared to skin from control subjects, such as iNOS, TNF-α, IL-10, IL-22 and IL-23. These cytokines play an important role in the inflammation observed in psoriasis and are markedly increased in both biopsies areas, affected and non-affected skin. TNF-α has a recognized role in lesions associated with psoriasis, as well as IL-22 and IL-23.

The importance of these modulators in triggering lesions of psoriasis can be demonstrated by the therapeutic effects of their blockages by immunobiological drugs, as observed for TNF-α, IL-23 and IL-17. They are cytokines that promote and support the production of IFN-γ and IL-17 and are also associated with the hyperproliferation of keratinocytes, with the production of antimicrobial peptides and with the influx of neutrophils (EYERICH *et al.*, 2009; WOLK *et al.*, 2006). IL-10 on its side, represents a regulatory cytokine found in the skin lesion and produced by PBMC. However, its production does not seem to be enough to control the inflammatory process (FURIATI *et al.*, 2019).

RNA expression of iNOS showed higher levels in patients with psoriasis than in control subjects, suggesting the participation of this cell population and of inflammatory mediators of nitric oxide in the pathogenesis of psoriatic lesions (LOWES *et al.*, 2005). Previous studies have reported higher levels of mRNA expression of iNOS in biopsies of

affected skin from patients with psoriasis than in the skin of healthy individuals (ORMEROD *et al.*, 1998; SIRSJÖ *et al.*, 1996).

Relative mRNA expression of GLUT1 was found to be significantly higher in the skin of patients with psoriasis than in the skin of healthy control subjects. GLUT1 is associated with high glucose intake and increased cellular metabolism. Studies have already suggested that GLUT1 can play a role in the pathogenesis of psoriasis through activation and differentiation of T cells into Th1 and Th17. Increased expression of GLUT1 indicates an increase in metabolism, both in immune cells and in keratinocytes (ABDOU *et al.*, 2013; HODEIB *et al.*, 2018; PALMER *et al.*, 2015).

The expression of the mRNA of these mediators was also analyzed based on the treatment. Twenty-one patients with psoriasis were analyzed, six of them were not undergoing any treatment and fifteen were receiving methotrexate, but with poor therapeutic response. In these patients, mRNA expression for ARG-2 IFN-γ and IL-10 was higher in non-affected skin biopsies of treated patients than in biopsies of the untreated ones. In turn, the expression of TGF-βR was greater in biopsies of affected skin in treated patients. These results reinforce the role of these mediators in the inflammatory process and in cell proliferation observed in psoriasis, demonstrating that the low response to methotrexate is associated with an increased expression of these mediators, even when compared to their expressions in untreated patients. Low expression of TGF-βR1 and TGF-βR2 receptors has been observed previously (DOI *et al.*, 2003; LEIVO *et al.*, 1998). Its high expression observed in this study suggests that it may be more involved with cell proliferation than with immunoregulation.

CONCLUSION

An increase in inflammation mediators was observed in skin biopsies of patients with psoriasis when compared with the control group in both affected and non-affected skin samples. Furthermore, the paradoxical increased expression of IFN-γ, IL-17, TGF-βR, ARG1 and ARG2 in the affected tissue indicate the concomitant of an inflammatory and proliferative reaction.

REFERENCES

ABDOU, Asmaa G. *et al.* Immunohistochemical Expression of GLUT-1 and Ki-67 in Chronic Plaque Psoriasis. *The American Journal of Dermatopathology*, v. 35, n. 7, p. 731–737, out. 2013. Disponível em: <http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00000372-201310000-00004>.

ASADULLAH, K *et al.* IL-10 is a key cytokine in psoriasis. Proof of principle by IL-10 therapy: a new therapeutic approach. *Journal of Clinical Investigation*, v. 101, n. 4, p. 783–794, fev. 1998.

BREMBILLA, Nicolo Costantino; SENRA, Luisa; BOEHNCKE, Wolf-Henning. The IL-17 Family of Cytokines in Psoriasis: IL-17A and Beyond. *Frontiers in Immunology*, v. 9, n. AUG, 2 ago. 2018. Disponível em: <https://www.frontiersin.org/article/10.3389/fimmu.2018.01682/full>.

BRUCH-GERHARZ, Daniela; RUZICKA, Thomas; KOLB-BACHOFEN, V. Nitric oxide and its implications in skin homeostasis and disease - a review. *Archives of Dermatological Research*, v. 290, n. 12, p. 643–651, 14 dez. 1998. Disponível em: <http://link.springer.com/10.1007/s004030050367>.

DIZIKES, George J. *et al.* Isolation of human liver arginase cdna and demonstration of nonhomology between the two human arginase genes. *Biochemical and Biophysical Research Communications*, v. 141, n. 1, p. 53–59, nov. 1986.

DOI, Hisao *et al.* Downregulation of TGFβ isoforms and their receptors contributes to keratinocyte hyperproliferation in psoriasis vulgaris. *Journal of Dermatological Science*, v. 33, n. 1, p. 7–16, out. 2003. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0923181103001075>.

EYERICH, Stefanie *et al.* Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *Journal of Clinical Investigation*, nov. 2009.

FURIATI, Sandro C. *et al.* Th1, Th17, and Treg Responses are Differently Modulated by TNF-α Inhibitors and Methotrexate in Psoriasis Patients. *Scientific Reports*, v. 9, n. 1, p. 7526, 17 dez. 2019. Disponível em: <http://www.nature.com/articles/s41598-019-43899-9>.

GHERZI, Roberto *et al.* “HepG2/Erythroid/Brain” type glucose transporter (GLUT1) is

highly expressed in human epidermis: Keratinocyte differentiation affects glut1 levels in reconstituted epidermis. *Journal of Cellular Physiology*, v. 150, n. 3, p. 463–474, mar. 1992.

GRINE, Lynda *et al.* An inflammatory triangle in psoriasis: TNF, type I IFNs and IL-17. *Cytokine & Growth Factor Reviews*, v. 26, n. 1, p. 25–33, fev. 2015. Disponível em: <http://dx.doi.org/10.1016/j.cytogfr.2014.10.009>.

HARRINGTON, Laurie E. *et al.* Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nature immunology*, v. 6, n. 11, p. 1123–32, 2 nov. 2005. Disponível em: <http://www.nature.com/articles/ni1254>.

HAWKES, Jason E. *et al.* Discovery of the IL-23/IL-17 Signaling Pathway and the Treatment of Psoriasis. *The Journal of Immunology*, v. 201, n. 6, p. 1605–1613, 15 set. 2018. Disponível em: <http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1800013>.

HODEIB, Abeer A.H. *et al.* Glucose transporter-1 (GLUT-1) expression in psoriasis: correlation with disease severity. *International Journal of Dermatology*, v. 57, n. 8, p. 943–951, ago. 2018. Disponível em: <http://doi.wiley.com/10.1111/ijd.14037>.

HOMEY, Bernhard *et al.* Up-Regulation of Macrophage Inflammatory Protein-3α/CCL20 and CC Chemokine Receptor 6 in Psoriasis. *The Journal of Immunology*, v. 164, n. 12, p. 6621–6632, jun. 2000.

JACOB, Sharon E. *et al.* Simultaneous measurement of multiple Th1 and Th2 serum cytokines in psoriasis and correlation with disease severity. *Mediators of Inflammation*, v. 12, n. 5, p. 309–313, 2003. Disponível em: <http://www.hindawi.com/journals/mi/2003/735813/abs/>.

JIAWEN, Li; DONGSHENG, Li; ZHIJIAN, Tan. The expression of interleukin-17, interferon-gamma, and macrophage inflammatory protein-3 alpha mRNA in patients with psoriasis vulgaris. *Journal of Huazhong University of Science and Technology [Medical Sciences]*, v. 24, n. 3, p. 294–296, jun. 2004.

KEPKA-LENHART, Diane *et al.* Arginase I: a limiting factor for nitric oxide and polyamine synthesis by activated macrophages? *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, v. 279, n. 6, p. R2237–R2242, dez. 2000.

KIMURA, A.; NAKA, T.; KISHIMOTO, T. IL-6-dependent and -independent pathways in the development of interleukin 17-producing T helper cells. *Proceedings of the National Academy of Sciences*, v. 104, n. 29, p. 12099–12104, jul. 2007.

KRYCZEK, Ilona *et al.* Induction of IL-17 + T Cell Trafficking and Development by IFN-γ: Mechanism and Pathological Relevance in Psoriasis. *The Journal of Immunology*, v. 181, n. 7, p. 4733–4741, 1 out. 2008. Disponível em: <http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.181.7.4733>.

LEIVO *et al.* Down-regulation of transforming growth factor-beta receptors I and II is seen in lesional but not non-lesional psoriatic epidermis. *British Journal of Dermatology*, v. 138, n. 1, p. 57–62, jan. 1998.

LI, Hui *et al.* Regulatory role of arginase I and II in nitric oxide, polyamine, and proline syntheses in endothelial cells. *American Journal of Physiology-Endocrinology and Metabolism*, v. 280, n. 1, p. E75–E82, jan. 2001.

LIVAK, Kenneth J.; SCHMITTGEN, Thomas D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods*, v. 25, n. 4, p. 402–408, dez. 2001. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S1046202301912629>.

LOWES, M. A. *et al.* Increase in TNF- and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proceedings of the National Academy of Sciences*, v. 102, n. 52, p. 19057–19062, dez. 2005.

LYNDE, Charles W. *et al.* Interleukin 17A: Toward a new understanding of psoriasis pathogenesis. *Journal of the American Academy of Dermatology*, v. 71, n. 1, p. 141–150, jul. 2014.

MATTOZZI, Carlo *et al.* Importance of Regulatory T Cells in the Pathogenesis of Psoriasis: Review of the Literature. *Dermatology*, v. 227, n. 2, p. 134–145, 2013.

NESTLE, Frank O. *et al.* Skin immune sentinels in health and disease. *Nature Reviews Immunology*, v. 9, n. 10, p. 679–691, out. 2009.

NOGRALES, K.E. *et al.* Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. *British Journal of Dermatology*, ago. 2008.

ORMEROD, A. D. *et al.* Detection of nitric oxide and nitric oxide synthases in psoriasis. *Archives of Dermatological Research*, v. 290, n. 1–2, p. 3–8, jan. 1998.

PALMER, Clovis S. *et al.* Glucose Metabolism Regulates T Cell Activation, Differentiation, and Functions. *Frontiers in Immunology*, v. 6, jan. 2015.

PATEL, Dhavalkumar D *et al.* Effect of IL-17A blockade with secukinumab in autoimmune diseases. *Annals of the Rheumatic Diseases*, v. 72, n. suppl 2, p. 116–123, abr. 2013.

RIVAS BEJARANO, Joaquin J.; VALDECANTOS, Wendell C. Psoriasis as Autoinflammatory Disease. *Dermatologic Clinics*, v. 31, n. 3, p. 445–460, jul. 2013.

RYAN, Sheila. Psoriasis: characteristics, psychosocial effects and treatment options. *British Journal of Nursing*, v. 17, n. 5, p. 284–290, mar. 2008.

SCHÖN, Michael P.; BOEHNCKE, W.-Henning. Psoriasis. *New England Journal of Medicine*, v. 352, n. 18, p. 1899–1912, maio 2005.

SICA, Antonio; MANTOVANI, Alberto. Macrophage plasticity and polarization: in vivo veritas. *Journal of Clinical Investigation*, v. 122, n. 3, p. 787–795, mar. 2012.

SIRSJÖ, A. *et al.* Increased expression of inducible nitric oxide synthase in psoriatic skin and cytokine-stimulated cultured keratinocytes. *British Journal of Dermatology*, v. 134, n. 4, p. 643–648, abr. 1996. Disponível em: <http://doi.wiley.com/10.1111/j.1365-2133.1996.tb06963.x>.

TOCHIO, Takumi; TANAKA, Hiroshi; NAKATA, Satoru. Glucose transporter member 1 is involved in UVB-induced epidermal hyperplasia by enhancing proliferation in epidermal keratinocytes. *International Journal of Dermatology*, v. 52, n. 3, p. 300–308, mar. 2013.

WOLK, Kerstin *et al.* IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. *European Journal of Immunology*, v. 36, n. 5, p. 1309–1323, maio 2006.

ZABA, Lisa C. *et al.* Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *Journal of Experimental Medicine*, v. 204, n. 13, p. 3183–3194, 24 dez. 2007. Disponível em:<https://rupress.org/jem/article/204/13/3183/46570/Amelioration-of-epidermal-hyperplasia-by-TNF>.

Table 1. Gender and mean age of patients and healthy subjects.

	Gender (Male/Female)	**Age – mean (Male/Female)**
Healthy subjects	3/2	40/44,5
Patients without therapy	3/3	38,7/48
Patients treated with methotrexate	5/10	50,2/53

Figure 1. Psoriasis. Quantitative analysis of mRNA levels by qRT-PCR for IL-17, IFN-γ, IL-23, IL-22, IL-10 and TNF-α in biopsies of affected and non-affected tissue from patients with psoriasis in comparison with healthy tissue. (A) Relative expression of IL-17; (B) Relative expression of IFN-γ; (C) Relative expression of IL-23; (D) Relative expression of IL-22; (E) Relative expression of IL-10; (F) Relative expression of TNF-α. For the analysis between three groups, the Kruskal-Wallis test was used. For the analysis between two groups with dependent variables, the Wilcoxon test was used. *Statistically significant difference.

Figure 2. Psoriasis. Quantitative analysis of mRNA levels by qRT-PCR for TGF-βR2, ARG1, ARG2, iNOS and GLUT1 in biopsies of affected and non-affected tissue from patients with psoriasis in comparison with healthy tissue. (A) Relative expression of TGF-βR2; (B) Relative expression of ARG1; (C) Relative expression of ARG2; (D) Relative expression of iNOS; (E) Relative expression of GLUT1. For the analysis between three groups, the Kruskal-Wallis test was used. For the analysis between two groups with dependent variables, the Wilcoxon test was used. *Statistically significant difference.

Figure 3. Psoriasis. Quantitative analysis of mRNA levels by qRT-PCR for ARG2, IFN-γ, Il-10 and TGF-βR2 in biopsies of affected and non-affected tissue from patients with psoriasis undergoing methotrexate therapy and untreated patients. (A) Relative expression of ARG2; (B) Relative expression of IFN-γ; (C) Relative expression of IL-10; (D) (Mann-Whitney $p < 0.05$). The Mann-Whitney test was used for analysis between two independent groups. *Statistically significant difference.

Figure 1

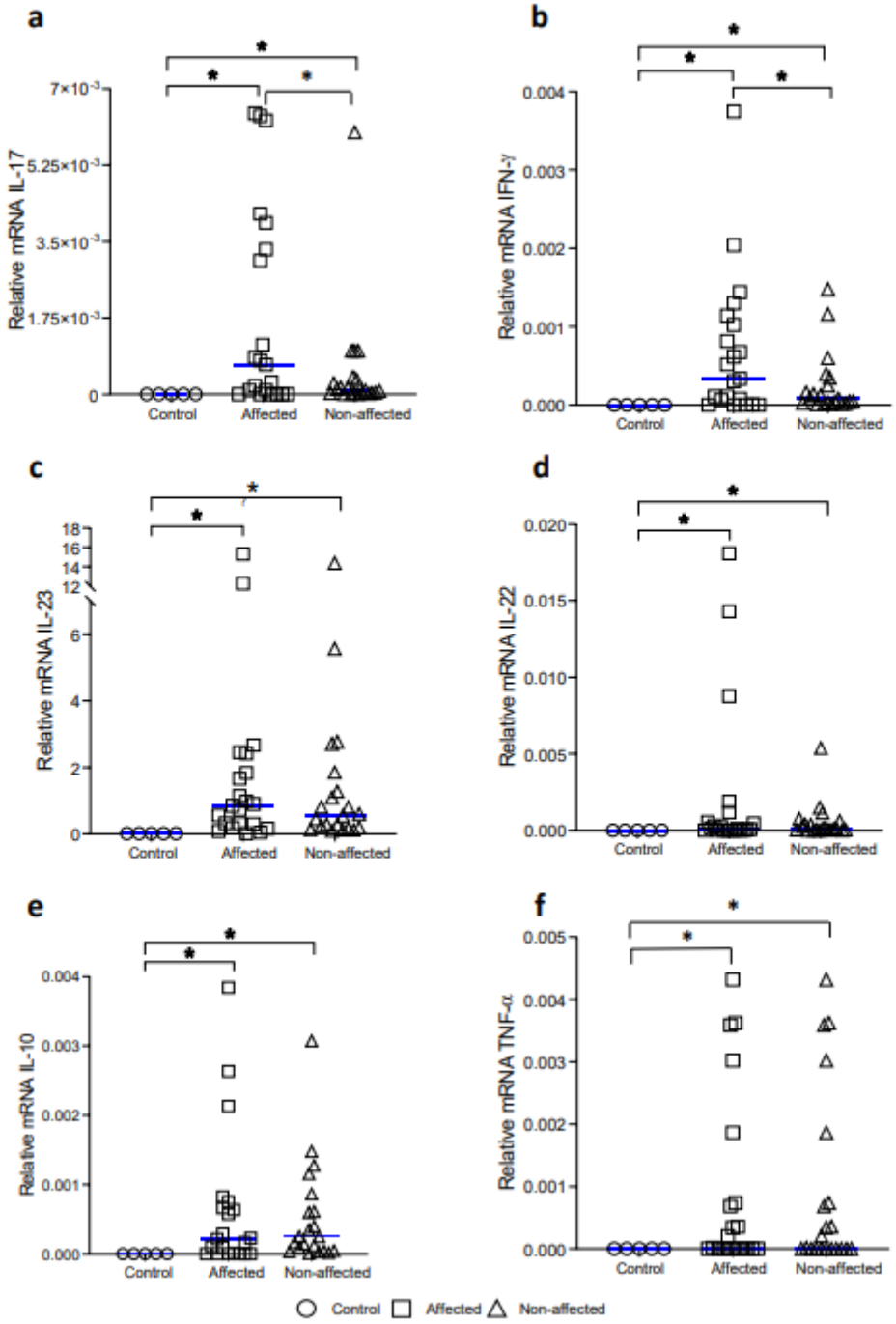

a
Relative mRNA IL-17
7×10⁻³
5.25×10⁻³
3.5×10⁻³
1.75×10⁻³
0
Control
Affected
Non-affected
b
Relative mRNA IFN-γ
0.004
0.003
0.002
0.001
0.000
c
Relative mRNA IL-23
18
16
14
12
6
4
2
d
Relative mRNA IL-22
0.020
0.015
0.010
0.005
e
Relative mRNA IL-10
f
Relative mRNA TNF-α
Control
Affected
Non-affected

Figure 2

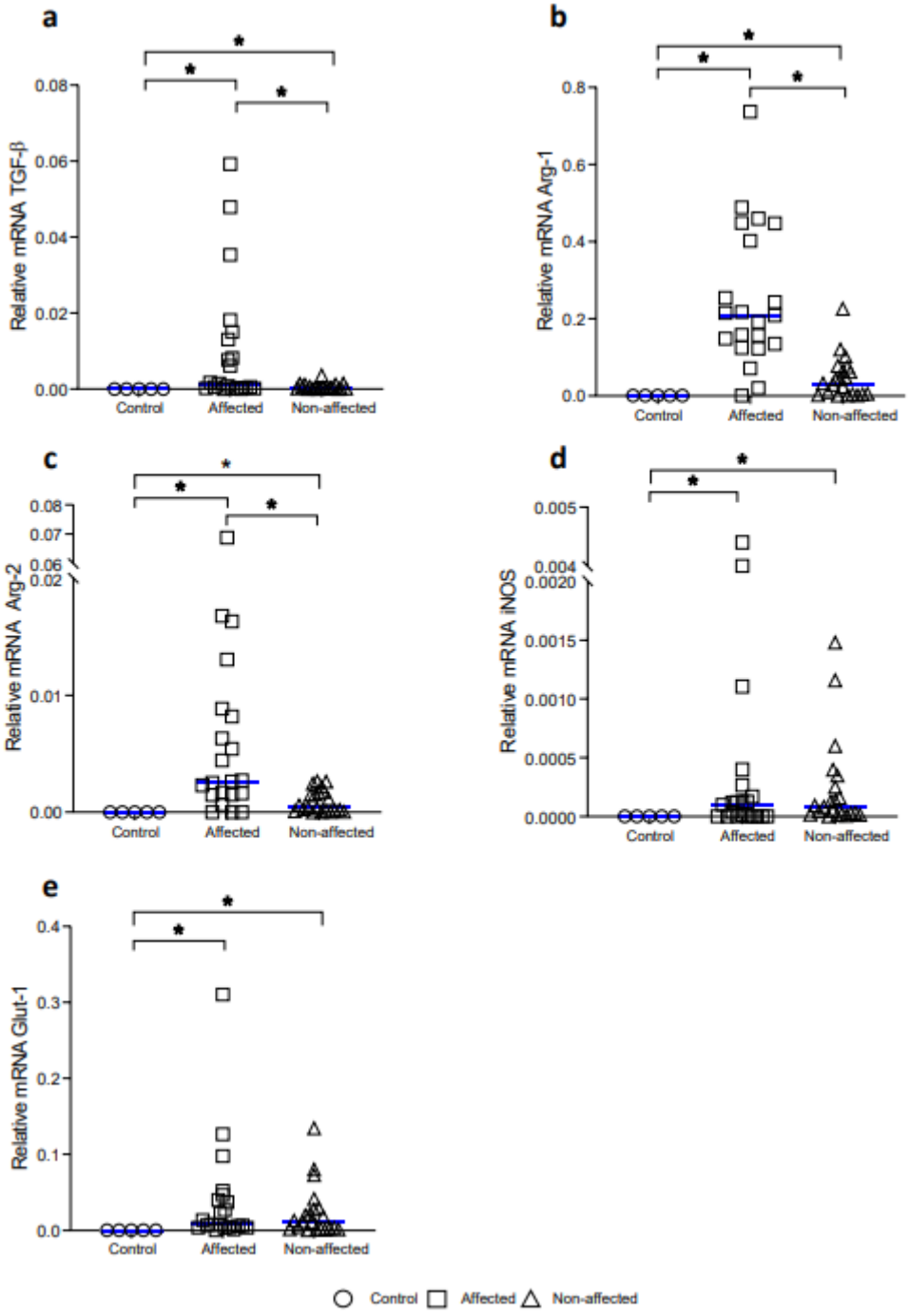
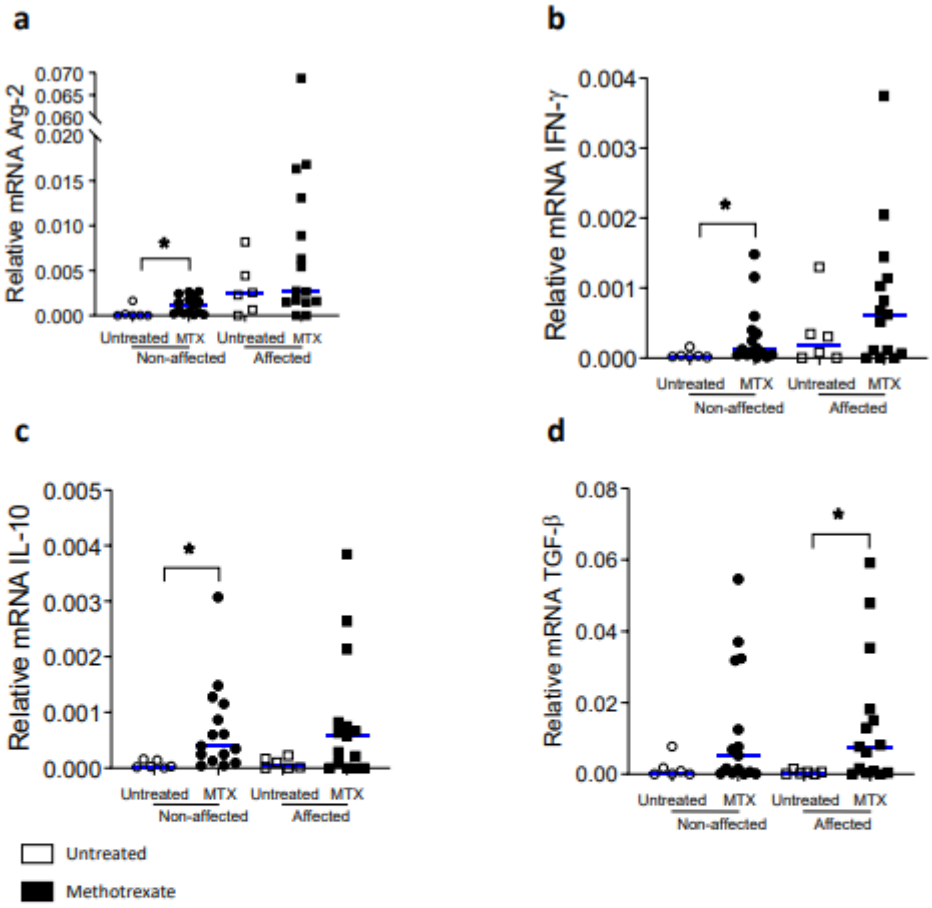

a
Relative mRNA TGF-β
0.08
0.06
0.04
0.02
0.00
Control
Affected
Non-affected
b
Relative mRNA Arg-1
0.8
0.6
0.4
0.2
0.0
Control
Affected
Non-affected
c
Relative mRNA Arg-2
0.08
0.07
0.06
0.02
0.01
0.00
Control
Affected
Non-affected
d
Relative mRNA iNOS
0.005
0.004
0.0020
0.0015
0.0010
0.0005
0.0000
Control
Affected
Non-affected
e
Relative mRNA Glut-1
0.4
0.3
0.2
0.1
0.0
Control
Affected
Non-affected
Control
Affected
Non-affected

Figure 3


a
Relative mRNA Arg-2
0.070
0.065
0.060
0.020
0.015
0.010
0.005
0.000
*
Untreated MTX Untreated MTX
Non-affected Affected
b
Relative mRNA IFN-γ
0.004
0.003
0.002
0.001
0.000
*
Untreated MTX Untreated MTX
Non-affected Affected
c
Relative mRNA IL-10
0.005
0.004
0.003
0.002
0.001
0.000
*
Untreated MTX Untreated MTX
Non-affected Affected
d
Relative mRNA TGF-β
0.08
0.06
0.04
0.02
0.00
*
Untreated MTX Untreated MTX
Non-affected Affected
Untreated
Methotrexate