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REATIVIDADE SOROLÓGICA ANTI-*Toxocara* EM DOENÇAS REUMÁTICAS AUTOIMUNES REVELA SUPRESSÃO SELETIVA DE IGG4 NO LÚPUS ERITEMATOSO SISTÊMICO

LARA CARDOSO RABELLO

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Dissertação apresentada ao Colegiado do Programa de Pós-Graduação em Imunologia e Parasitologia Aplicadas como parte de obtenção do título de Mestre

Lara Cardoso Rabello

Orientador Prof. Dr. Rodrigo Rodrigues Cambraia de Miranda

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RESUMO

A exposição a helmintos tem sido sugerida como um fator modulador da inflamação autoimune, porém ainda há lacunas quanto à caracterização da resposta imune anti-helmíntica em doenças reumáticas autoimunes. Neste estudo, analisamos os perfis da sororreatividade anti-*Toxocara canis* em indivíduos com artrite reumatoide (AR), lúpus eritematoso sistêmico (LES) e espondiloartrites (EpA), em comparação com controles pareados, além de investigar possíveis associações clínicas. Foi realizado um estudo transversal do tipo caso-controle, incluindo 442 participantes. Inicialmente, as amostras foram testadas para IgG anti-*T. canis* utilizando antígeno TES nativo e as amostras reativas foram posteriormente avaliadas com o antígeno quimérico recombinante rSHORT para detecção de IgG total, IgG1 e IgG4. A atividade da doença foi mensurada pelo SLEDAI no LES, e a incapacidade funcional pelo HAQ-DI. Os resultados demonstraram alta frequência de sororreatividade anti-*Toxocara* na população estudada. Após ajustes para variáveis demográficas, pacientes com LES apresentaram maior chance de positividade para IgG total em relação aos controles, enquanto a sororreatividade para IgG4 mostrou-se significativamente reduzida. Não foram observadas associações independentes entre os isotipos de IgG e o HAQ-DI em pacientes com AR ou EpA. No grupo com LES, níveis mais elevados de eosinófilos associaram-se de forma independente à menor atividade da doença. Em conjunto, os achados evidenciam padrões heterogêneos de resposta sorológica anti-*T. canis* nas doenças reumáticas autoimunes, destacando-se o LES por apresentar manutenção da resposta de IgG total concomitante a uma redução seletiva da sororreatividade IgG4. Além disso, os eosinófilos, e não a IgG4, mostraram-se mais fortemente relacionados à menor atividade da doença no LES.

Palavras-chave: *Toxocara canis*; doenças reumáticas autoimunes; lúpus eritematoso sistêmico; IgG4; eosinófilos.

ABSTRACT

Helminth exposure has been suggested as a modulating factor in autoimmune inflammation; however, gaps remain regarding the quality of anti-helminth immune responses in autoimmune rheumatic diseases. In this study, we analyzed anti-*Toxocara canis* seroreactivity profiles in individuals with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and spondyloarthritis (SpA), compared with controls, and also investigated potential clinical associations. A cross-sectional case-control study was conducted, including 442 participants. Initially, samples were tested for anti-*T. canis* IgG using native TES antigen, and reactive samples were subsequently evaluated using the recombinant chimeric antigen rSHORT for the detection of total IgG, IgG1, and IgG4. Disease activity was assessed using SLEDAI in SLE, and functional disability using HAQ-DI. The results demonstrated a high frequency of anti-*T. canis* seroreactivity in the studied population. After adjustment for demographic variables, patients with SLE showed higher odds of total IgG positivity compared with controls, whereas IgG4 seroreactivity was significantly reduced. No independent associations were observed between IgG isotypes and HAQ-DI in patients with RA or SpA. Within the SLE group, higher eosinophil levels were independently associated with lower disease activity. Overall, these findings indicate heterogeneous patterns of anti-*Toxocara* serological responses in autoimmune rheumatic diseases, with SLE showing the most distinctive profile, characterized by preserved total IgG responses alongside reduced IgG4 seroreactivity. Furthermore, eosinophils, rather than IgG4, were more strongly associated with lower disease activity in SLE.

Keywords: *Toxocara canis*; autoimmune rheumatic diseases; systemic lupus erythematosus; IgG4; eosinophils

LISTA DE ABREVIATURAS E SIGLAS

ABZ – Albendazol

ACR – American College of Rheumatology

ANA – Anticorpos antinucleares

AP – Artrite psoriásica

AR – Artrite reumatoide

DCs – Células dendríticas

DNA – Ácido desoxirribonucleico

EA – Espondilite anquilosante

ESSG – Grupo Europeu de Estudo da Espondiloartropatia

ELISA – Enzyme-Linked Immunosorbent Assay (ensaio imunoenzimático)

EpA – Espondiloartrite

EpA-DII – Artrite associada à doença inflamatória intestinal

EpA-I – Espondiloartrite indiferenciada

HC-UFU – Hospital de Clínicas da Universidade Federal de Uberlândia

IFN-γ – Interferon gama

IgG – Imunoglobulina G

IgG4 – Subclasse 4 da imunoglobulina G

IL-4 – Interleucina 4

IL-5 – Interleucina 5

IL-6 – Interleucina 6

IL-10 – Interleucina 10

IL-12 – Interleucina 12

IL-13 – Interleucina 13

IL-17 – Interleucina 17

LADECH – Laboratório de Diagnóstico, Epidemiologia e Controle de Helmintos

L2 – Larva de segundo estágio

L3 – Larva de terceiro estágio

L4 – Larva de quarto estágio

LES – Lúpus eritematoso sistêmico

MBZ – Mebendazol

rSHORT – Proteína recombinante quimérica SHORT

TBZ – Tiabendazol

TES – Antígeno excretor-secretor de *Toxocara*

TGF-β – Fator de crescimento transformador beta

Th1 – Linfócitos T auxiliares do tipo 1

Th2 – Linfócitos T auxiliares do tipo 2

Th17 – Linfócitos T auxiliares do tipo 17

Treg / Tregs – Células T reguladoras

UFBA – Universidade Federal da Bahia

UFU – Universidade Federal de Uberlândia

SUMÁRIO

APRESENTAÇÃO

Doenças autoimunes são enfermidades inflamatórias crônicas decorrentes da quebra da tolerância imunológica. Nesse contexto, células T e anticorpos passam a reagir contra células próprias e antígenos teciduais, resultando em perda ou limitação da função dos tecidos. Essas doenças apresentam um amplo espectro, com quase 100 enfermidades de base autoimune descritas, incluindo artrite reumatoide (AR), lúpus eritematoso sistêmico (LES) e espondiloartrite (EpA).

Toxocara spp. compreende nematódeos ascarídeos de cães e gatos, sendo *T. canis* e *T. cati* as principais espécies associadas à toxocaríase humana. Em humanos, a infecção ocorre de forma acidental, principalmente pela ingestão de ovos embrionados contendo larvas de terceiro estágio (L3) presentes no ambiente, ou pela ingestão de carne crua ou malcozida de hospedeiros infectados. As manifestações clínicas da toxocaríase humana são causadas pela migração autolimitante das larvas em diferentes órgãos. Essa migração pode ser assintomática ou resultar em uma ampla variedade de sinais e sintomas clínicos incluindo larvas migrans visceral e larva migrans ocular, neurotoxocaríase e toxocaríase oculta ou comum. Estimativas epidemiológicas atuais, baseadas em meta-análises, indicam uma soroprevalência global de aproximadamente 19%.

A hipótese da higiene e a hipótese dos “velhos amigos” buscam explicar a relação entre a incidência de doenças alérgicas e autoimunes, como asma, psoríase, artrite reumatoide e doenças inflamatórias do trato gastrointestinal, e a exposição a parasitos na população humana. De acordo com essas hipóteses, o contato com determinados microrganismos e helmintos ao longo da evolução teria desempenhado um papel fundamental na modulação e no desenvolvimento adequado do sistema imunológico.

A exposição a esses organismos, especialmente em fases precoces da vida, contribuiria para a “educação” do sistema imune, promovendo respostas regulatórias capazes de prevenir reações exacerbadas associadas a alergias e doenças autoimunes. Embora essas teorias não elucidem completamente os mecanismos envolvidos, evidências epidemiológicas indicam que populações com maior exposição a parasitos apresentam menor prevalência de doenças alérgicas e autoimunes, sugerindo uma possível relação protetora.

Diversos estudos têm demonstrado que helmintos intestinais são capazes de modular a microbiota intestinal e induzir efeitos imunorregulatórios que influenciam a progressão de doenças inflamatórias e autoimunes. Esses organismos podem promover um ambiente imunológico mais tolerogênico, contribuindo para a atenuação de respostas inflamatórias

exacerbadas.

Um dos principais mecanismos envolvidos é a indução de células T reguladoras (Tregs), células B reguladoras (Bregs), macrófagos alternativamente ativados e células dendríticas com perfil regulador que desempenham papel central na manutenção da tolerância imunológica. A ativação dessas células, associada ao aumento de mediadores anti-inflamatórios, como IL-10 e TGF-β e ao favorecimento de respostas humorais regulatórias, como a produção de IgG4, pode reduzir a ativação de células efetoras, bem como a produção e a circulação de autoanticorpos, considerados fatores-chave na patogênese de doenças autoimunes, sugerindo um potencial efeito protetor mediado pela infecção helmíntica.

O diagnóstico imunológico da toxocaríase humana baseia-se na detecção de anticorpos anti-*Toxocara canis,* geralmente por meio de ensaio imunoenzimático (ELISA) e/ou Western Blotting. A utilização do antígeno excretor-secretor de *Toxocara* (TES) é amplamente descrita na literatura; entretanto, sua limitada especificidade, especialmente pela possibilidade de reações cruzadas com outros helmintos, pode comprometer a interpretação dos resultados. Dessa forma, torna-se necessária a utilização de ferramentas diagnósticas mais específicas, capazes de reduzir reações cruzadas com antígenos nativos e evitar mascaramento de padrões sorológicos.

A presente dissertação foi realizada no Laboratório de Diagnóstico, Epidemiologia e Controle de Helmintos (LADECH), localizado na Universidade Federal de Uberlândia (UFU) com a colaboração da equipe médica do Setor de Reumatologia do Hospital de Clínicas de Uberlândia (HC-UFU), do Laboratório de Alergia e Acarologia da Universidade Federal da Bahia (UFBA), Universidade Federal de Pelotas e Universidade Federal do Rio Grande.

O objetivo deste estudo foi investigar a modulação da resposta imune a *T. canis* em pacientes com doenças reumáticas autoimunes, por meio da utilização de uma nova proteína recombinante quimérica (rSHORT). Partiu-se da hipótese de que componentes associados à resposta anti-helmíntica regulatória, incluindo IgG4 e eosinófilos, poderiam estar alterados em pacientes com doenças reumáticas autoimunes e que a preservação desses componentes poderia estar associada a desfechos clínicos mais favoráveis.

A presente dissertação está estruturada em dois capítulos, conforme descrito a seguir:

Capítulo 1 – Fundamentação teórica: apresenta uma revisão atualizada da literatura acerca de *Toxocara canis* e doenças autoimunes reumáticas.

Capítulo 2 – Seroreactivity to *Toxocara canis* antigens across autoimmune rheumatic diseases reveals selective IgG4 suppression in systemic lupus erythematosus: descreve os achados experimentais do estudo, com foco nos perfis de sororreatividade anti-*Toxocara canis* em doenças reumáticas autoimunes e em seus correlatos imunológicos e clínicos, incluindo IgG4, eosinófilos e atividade da doença.

CAPÍTULO 1 – FUNDAMENTAÇÃO TEÓRICA

1. INTRODUÇÃO

1.1. Toxocara canis e a toxocaríase humana

Toxocara canis é um nematódeo pertencente à família Ascarididae, tendo cães como principais hospedeiros. Seu ciclo de vida inclui diferentes estágios de desenvolvimento, compreendendo ovos não embrionados, embrionados, larvas de segundo, terceiro e quarto estágios (L2, L3 e L4) e o verme adulto (Moreira *et al*., 2014).

A infecção canina é mais frequente em cadelas prenhas, lactantes e filhotes, sendo a principal forma de infecção a transmissão transplacentária e transmamária (Felix *et al*., 2020). Os vermes adultos habitam o lúmen do intestino delgado, onde atingem a maturação sexual. Os ovos produzidos pelas fêmeas adultas passam pelo trato intestinal e são eliminados nas fezes como ovos não embrionados e não infectantes.

No ambiente, desenvolvem-se até o estágio infectante (L3) no solo, dependendo de condições climáticas como temperatura e umidade, tornando-se ovos embrionados. Após a ingestão desses ovos, ocorre a eclosão no intestino, com liberação das larvas, que penetram a mucosa intestinal e alcançam a circulação sanguínea.

As larvas migram pela via sangue–fígado–pulmão, onde se transformam em larvas L4 após penetrarem nos alvéolos pulmonares. Em seguida, migram para a traqueia, são deglutidas e alcançam o lúmen do duodeno como adultos imaturos. Alternativamente, larvas L3 também podem ser transmitidas por meio de hospedeiros paratênicos ou verticalmente entre cadelas e filhotes. Quando hospedeiros paratênicos ingerem ovos infectantes, o desenvolvimento larval ocorre apenas até o estágio L3, permanecendo inativo nos tecidos (Epe, 2009).

A toxocaríase humana é uma parasitose causada por *T. canis* e *T. cati*, cujos hospedeiros definitivos são cães e gatos. A doença apresenta distribuição cosmopolita, sendo mais prevalente em países de clima tropical e subtropical. A soroprevalência global estimada da toxocaríase humana é de aproximadamente 19%, estando associada a fatores geográficos, climáticos e socioeconômicos (Ma *et al*., 2020).

Os seres humanos adquirem a infecção principalmente pela ingestão de ovos embrionados presentes no solo ou em alimentos contaminados, bem como pela ingestão de larvas encapsuladas em tecidos de hospedeiros paratênicos que não foram submetidos a tratamentos térmicos, como bovinos, ovinos e aves. Após a ingestão, as larvas provenientes de ovos

infectantes eclodem no intestino delgado ou, no caso de hospedeiros paratênicos, permanecem encapsuladas nos tecidos. Em seguida, atravessam a parede intestinal, alcançam a circulação sistêmica e migram para diferentes órgãos, onde desencadeiam uma resposta inflamatória significativa. Geralmente, a infecção humana é assintomática ou apresenta sintomas leves e inespecíficos. No entanto, *T. canis* pode causar patologias, manifestando-se em quatro formas clínicas principais: larva migrans visceral, larva migrans ocular, toxocaríase comum e neurotoxocaríase, as quais podem levar a graves complicações à saúde (Chen *et al*., 2018).

1.2. Diagnóstico da toxocaríase

O diagnóstico da toxocaríase baseia-se em evidências clínicas, radiográficas e laboratoriais da doença. Geralmente, é realizado a partir da análise do histórico do paciente, exame clínico, exame microscópico direto de tecidos e exames sanguíneos, além da utilização de métodos sorológicos e moleculares (Chen *et al*., 2018). Diante das dificuldades associadas ao diagnóstico e ao tratamento de pacientes, torna-se necessária a adoção de novas abordagens que contribuam para o controle da toxocaríase humana. Os avanços em ferramentas de imunodiagnóstico, especialmente aqueles centrados em antígenos recombinantes, parecem representar a abordagem mais promissora para o desenvolvimento de novos ensaios diagnósticos para a toxocaríase humana (Moreira *et al.,*2014).

1.3 Resposta imune anti-helmíntica e imunomodulação induzida por helmintos

A toxocaríase aguda é caracterizada por níveis elevados das citocinas IL-10 e IL-4, associados à regulação negativa de IL-12 e de linfócitos do tipo Th1. Em estudos experimentais com camundongos, foi observada alta transcrição de IL-10 entre 24 e 48 horas após a infecção, indicando uma resposta imune precoce capaz de antagonizar a expressão de IL-12 (Borchard *et al.,* 2022).

As infecções crônicas por *T. canis* estão associadas ao aumento de anticorpos específicos e inespecíficos de diferentes isotipos de IgG, além de uma resposta celular do tipo Th2, caracterizada pela produção de IL-4, IL-5 e IL-13 (Shayesteh *et al*., 2020). Esse padrão imunológico evidencia o potencial imunomodulador do parasito, relacionado à indução de células dendríticas reguladoras, à ativação de células T reguladoras (Treg) e ao aumento da produção de citocinas anti-inflamatórias, como IL-10 e TGF-β.

1.4 Tratamento da toxocaríase humana

O tratamento da toxocaríase humana deve ser individualizado de acordo com a forma clínica e a gravidade do quadro. A conduta terapêutica varia conforme a síndrome apresentada, incluindo formas viscerais, neurológicas, oculares e formas com manifestações inespecíficas, de modo que a decisão de tratar deve considerar a intensidade dos sintomas, o órgão acometido e o risco de sequelas. Nos casos sintomáticos, os benzimidazóis constituem a base da terapia antiparasitária, com destaque para o albendazol, considerado atualmente a principal opção terapêutica em razão de sua ampla disponibilidade, baixo custo, perfil de segurança favorável e eficácia adequada. O mebendazol pode ser utilizado como alternativa, enquanto o tiabendazol e a dietilcarbamazina apresentam uso mais limitado, seja por menor tolerabilidade, seja por menor utilização na prática atual.

A duração do tratamento ainda não é completamente padronizada, havendo estudos com esquemas curtos, como 5 dias, e outros com períodos mais prolongados, de até 2 semanas ou mais, especialmente em apresentações específicas. De modo geral, recomenda-se albendazol em pacientes sintomáticos, embora permaneça controversa a necessidade de tratamento antiparasitário nos casos assintomáticos.

Nas formas em que a resposta inflamatória contribui substancialmente para a lesão tecidual, o uso de corticosteroides assume papel importante. Segundo Lopez-Alamillo et al. (2025), os corticosteroides devem preceder e acompanhar o uso de anti-helmínticos em situações como toxocaríase visceral com acometimento cardíaco ou eosinofilia periférica importante, toxocaríase ocular e neurotoxocaríase, com o objetivo de reduzir o recrutamento celular e a inflamação exacerbada induzidos durante o tratamento.

Assim, o tratamento da toxocaríase humana deve ser compreendido como uma abordagem integrada, envolvendo terapia antiparasitária, modulação da resposta inflamatória e manejo das complicações orgânicas, especialmente nas formas ocular e neurológica.

1.5 Doenças reumáticas autoimunes

As doenças reumáticas constituem um conjunto de condições inflamatórias que acometem as articulações e os tecidos conjuntivos, sendo frequentemente associadas à dor e à limitação da mobilidade (Podolska *et al*., 2018). O grupo heterogêneo de doenças reumáticas autoimunes inclui artrite reumatoide (AR) lúpus eritematoso sistêmico (LES), síndrome de Sjögren, esclerodermia de início na idade adulta, espondiloartrite (EpA) e polimiosite (PM) (Joseph *et al*., 2010). A artrite reumatoide (AR) é caracterizada como uma doença autoimune

sistêmica relacionada a um quadro inflamatório crônico, capaz de comprometer não apenas as articulações, mas também diversos órgãos extra-articulares, como coração, rins, pulmões, trato gastrointestinal, olhos, pele e sistema nervoso.

A espondiloartrite (EpA) é um termo utilizado para designar um conjunto de doenças reumáticas inflamatórias crônicas que compartilham características fisiopatológicas, genéticas e clínicas em comum (Proft *et al*., 2024). Cinco subtipos principais de espondiloartrite (SpA) são reconhecidos com base nos critérios de classificação propostos pelo Grupo Europeu de Estudo da Espondiloartropatia (ESSG), a espondilite anquilosante (EA), artrite reativa (AR), artrite psoriásica (AP), artrite associada à doença inflamatória intestinal (EpA-DII) e espondiloartrite indiferenciada (EpA-I). (Sharip; Kunz *et al*,. 2020) As principais manifestações clínicas da EpA são dor inflamatória, rigidez e edema das articulações e da coluna vertebral (Stolwijk *et al*., 2012).

O lúpus eritematoso sistêmico (LES) é uma doença autoimune crônica, com uma ampla gama de manifestações clínicas e um curso caracterizado por períodos de remissão e recaídas (Zucchi *et. al*., 2023). O LES é descrito como uma doença caracterizada por hiperatividade das células B e produção policlonal de anticorpos (Tsokos *et. al*., 2011). Afeta aproximadamente 3,4 milhões de pessoas em todo o mundo, com 400.000 novos diagnósticos a cada ano (Tian *et. al*., 2022). O LES acomete principalmente entre mulheres entre a puberdade e a menopausa (Pons-Estel *et. al*., 2010).

Os sintomas iniciais na maioria dos pacientes são constitucionais, mucocutâneos e musculoesqueléticos, podendo incluir fadiga, erupção cutânea, úlceras na boca, alopecia, dor articular e mialgia (Hoi *et. al*., 2024). As manifestações clínicas são heterogêneas, variando de doença cutânea leve a falências de órgãos e complicações obstétricas (Barber *et. al.,* 2021).

O diagnóstico é desafiador e atualmente é realizado por meio de manifestações clínicas e exames laboratoriais, como detecção de autoanticorpos, testes funcionais e exames de imagem (Mosca *et al*., 2010). O critério de classificação ACR de 1997 tem sido amplamente utilizado, com os índices de classificação sendo eritema malar, eritema discoide, fotossensibilidade, úlceras orais, artrite não erosiva, pleurite ou pericardite, distúrbio renal, distúrbio neurológico, síndrome hematológica, evidência imunológica e ANA positivas (Hochberg, 1997).

O LES é causado por uma reação autoimune que envolve os sistemas imunológicos inatos e adaptativos, nos quais uma resposta imune anormal é direcionada a partículas celulares que contêm ácidos nucleicos, sendo que a superprodução de anticorpos direcionados a esses ácidos é uma característica da enfermidade (Kaul *et. al*., 2016).

Diversos agentes são utilizados no tratamento e, embora vários medicamentos tenham se

mostrado eficazes como hidroxicloroquina, diversos fármacos têm sido relatados como capazes de induzir o LES (Dai; Fan; Zhao, 2025). Esses fatores complicam a compreensão sobre o tratamento da doença, tornando-se necessário o aprofundamento de estudos.

1.6 Helmintos, hipótese da higiene/old friends e autoimunidade.

A hipótese dos "velhos amigos", originalmente formulada como hipótese da higiene, reconhece a importância de uma variedade de microrganismos, como helmintos e bactérias, para o desenvolvimento e regulação do sistema imunológico (Parker, 2014). Apesar de serem potencialmente patogênicos, esses organismos parecem desempenhar papel essencial no desenvolvimento, regulação e função imunológica.

Os helmintos, por possuírem propriedades imunomoduladoras, podem persistir nos hospedeiros por longos períodos, exercendo uma pressão evolutiva singular ao moldar as respostas imunes (Maizels; Smits; McSorley, 2018). Diversos estudos demonstram que helmintos intestinais podem alterar a microbiota intestinal e desencadear efeitos imunossupressores, influenciando a progressão de doenças inflamatórias e autoimunes (Osada, *et al*., 2013).

Devido à produção de anticorpos específicos contra autoantígenos e ao aumento das concentrações plasmáticas das citocinas IL-6 e IL-10, sugere-se que o lúpus eritematoso sistêmico (LES) apresente um perfil imunológico polarizado para Th2. No entanto, estudos demonstram que citocinas associadas à resposta Th1, incluindo IL-12 e interferon-γ, também se encontram elevadas em pacientes com LES (Praprotnik *et al.,* 2008). Além disso, evidências indicam que o aumento da expressão gênica de fatores de transcrição relacionados aos perfis Th1 e Th2 está correlacionada com a atividade da doença em pacientes com LES (Lit *et al*., 2007).

Diversos mecanismos estão envolvidos na imunomodulação induzida por parasitos, incluindo o uso de antígenos parasitários para modular o equilíbrio da resposta imunológica em direção a um perfil Th2, menos inflamatório e com papel regulador sobre respostas Th1 e Th17 (Alexandre-Silva *et al*., 2018). Sugere-se que os helmintos possam induzir uma resposta Th2 modificada juntamente com a expansão de células imunorreguladoras, como as Tregs, contribuindo para o controle da resposta imune do hospedeiro (Haspeslagh *et al*., 2018). Além disso, as infecções por helmintos podem promover imunomodulação por diferentes vias, incluindo a inibição de IFN-γ e IL-17, a indução de IL-4 e IL-10, a liberação do fator de crescimento transformador (TGF-β), a expressão de células T CD4+ FoxP3+ e geração de

células dendríticas reguladoras, macrófagos e células B (Grainger *et al.,* 2010).

OBJETIVOS

OBJETIVO GERAL

Investigar os perfis da resposta imune anti-*Toxocara canis* em pacientes com doenças reumáticas autoimunes, utilizando a proteína recombinante quimérica rSHORT, e avaliar seus correlatos clínicos

OBJETIVOS ESPECÍFICOS

Comparar a sororreatividade anti-*T. canis* (IgG, IgG1 e IgG4) entre pacientes com artrite reumatoide, lúpus eritematoso sistêmico e espondiloartrite e seus respectivos controles pareados;

Avaliar se componentes da resposta anti-helmíntica com perfil regulatório, especialmente IgG4 e eosinófilos, apresentam alterações em pacientes com autoimunidade;

Investigar a associação entre marcadores imunológicos anti-*T. canis* e parâmetros clínicos de atividade da doença e incapacidade funcional.

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CAPÍTULO 2 - SEROREACTIVITY TO *Toxocara canis* ANTIGENS ACROSS AUTOIMMUNE RHEUMATIC DISEASES REVEALS SELECTIVE IGG4 SUPPRESSION IN SYSTEMIC LUPUS ERYTHEMATOSUS

Title: Seroreactivity to *Toxocara canis* antigens across autoimmune rheumatic diseases reveals selective IgG4 suppression in systemic lupus erythematosus

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Abstract

Purpose: Helminth exposure has been proposed to modulate autoimmune inflammation, but the quality of anti-helminth immune responses in autoimmune rheumatic diseases remains unclear. We evaluated anti-*Toxocara* seroreactivity profiles in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and spondyloarthritis (SpA), compared

with corresponding control groups, and investigated their clinical correlates.

Methods: We conducted a cross-sectional case-control study including 442 individuals. All sera were initially screened for anti-*Toxocara* IgG using native TES antigen. TES-reactive samples were subsequently assessed with the recombinant chimeric antigen rSHORT for IgG, IgG1, and IgG4. Disease activity was evaluated with SLEDAI in SLE, and functional disability with HAQ-DI. Multivariable logistic regression models were adjusted for age, sex, and household income; disease activity in SLE was further examined using negative binomial regression.

Results: Anti-*Toxocara* IgG seroreactivity was frequent across the study population. In adjusted analyses, SLE was associated with higher odds of IgG seroreactivity than controls (OR 2.44, 95% CI 1.16–5.14; p=0.019), whereas IgG4 seroreactivity was significantly reduced (OR 0.20, 95% CI 0.04–0.99; p=0.049). No independent association of IgG, IgG1, or IgG4 with HAQ-DI was detected in RA or SpA after adjustment. Within SLE, higher eosinophil counts were independently associated with lower SLEDAI scores (p=0.008).

Conclusion: Autoimmune rheumatic diseases showed heterogeneous anti-*Toxocara* serological patterns. The most distinctive profile was observed in SLE, which combined preserved total IgG seroreactivity with reduced IgG4 seroreactivity. Within SLE, eosinophils, rather than IgG4, were the main independent correlate of lower disease activity.

Keywords: Helminth exposure; Eosinophils; Serology; Immune regulation; Autoimmune rheumatic diseases.

Introduction

Helminth infections and autoimmune diseases typically occupy opposite ends of the immunological spectrum. Helminths have co-evolved with humans to promote a regulatory immune environment, commonly characterized by type 2 responses, eosinophilia, and increased interleukin-10 (IL-10) and IgG4 production, whereas autoimmune diseases arise from the breakdown of tolerance and persistent inflammation [1,2]. In this context, the hygiene hypothesis proposes that the loss of ancestral exposure to immunoregulatory organisms, including helminths, may contribute to the increasing incidence of autoimmune disorders in industrialized settings [3,4].

Toxocara spp. infection is a neglected zoonotic helminthiasis of global importance [5,6]. Human infection occurs through ingestion of embryonated eggs from contaminated

environments or, less commonly, through consumption of raw or undercooked tissues from paratenic hosts or contaminated vegetables [6]. Humans are accidental hosts in whom larvae hatch in the intestine, penetrate the mucosa, enter the circulation, and migrate through somatic tissues without developing into adult worms. Although many infections remain asymptomatic or present with non-specific manifestations, this larval migration may result in clinically recognized syndromes such as visceral larva migrans, ocular larva migrans, covert toxocariasis, and neurotoxocariasis [7]. Because the parasite does not complete its life cycle in humans, eggs are not shed in stool, making coprological diagnosis uninformative. As a result, diagnosis relies largely on serology, although assays based on native antigens may show cross-reactivity with other helminth infections [8].

Current meta-analyses estimate a global seroprevalence of approximately 19%, corresponding to nearly 1.4 billion exposed or infected individuals worldwide [9]. In Brazil, reported seroprevalence rates vary widely, ranging from 4.2% to 65% depending on the population studied [10], and may be even higher in socially vulnerable settings, including indigenous communities [11]. Following infection, migrating larvae and their excretory-secretory antigens (TES) induce a systemic type 2 immune response characterized by eosinophilia and IgE production. In experimental models, this response can be granulomagenic and pathogenic [12,13]. In humans, chronic helminth infections have been associated with a more regulated immune profile [14], including pathways linked to IgG4 production [15].

The relationship between helminth infections and systemic lupus erythematosus (SLE) is particularly complex. Experimental studies and review data suggest that helminth-derived immunomodulation may delay lupus-like disease onset and attenuate nephritis in murine models, at least partly through regulatory pathways [16]. In contrast, human evidence remains limited and sometimes conflicting; although some observations support a protective effect, isolated reports have suggested that toxocariasis may overlap clinically with, or possibly precipitate, lupus manifestations in susceptible individuals [17]. These discrepancies highlight the need to investigate not only helminth exposure itself, but also the qualitative profile of the host immune response.

The interplay between helminth-driven immunoregulation and autoimmune rheumatic diseases (ARDs) remains unresolved. Although helminth-derived molecules can ameliorate arthritis in experimental models [18], the qualitative profile of anti-helminth immunity in human autoimmunity is still poorly understood. SLE is marked by B-cell hyperactivity and polyclonal antibody production [19], yet it is unknown whether regulatory components of the anti-helminth response, particularly IgG4, are preserved in this inflammatory environment.

The same uncertainty applies to rheumatoid arthritis (RA) and spondyloarthritis (SpA). Clarifying this issue requires highly specific serological tools, since native antigens may generate cross-reactivity and distort genuine response patterns [8].

In this study, we investigated the qualitative profile of anti-*Toxocara* immune responses in patients with ARDs using the recombinant chimeric antigen rSHORT [20], a highly specific serological tool designed to reduce cross-reactivity.

Materials and Methods

Study Design, Population, and Ethics

We conducted a cross-sectional case-control study including 442 individuals. Patients with autoimmune rheumatic diseases were recruited at the Rheumatology Outpatient Clinic of the Hospital de Clínicas de Uberlândia, Federal University of Uberlândia (HC-UFU), Uberlândia, Minas Gerais, Brazil. Control individuals without autoimmune rheumatic diseases were selected to achieve close comparability with cases with respect to age, sex, and household income. Patients were classified as having rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or spondyloarthritis (SpA) according to established international criteria: the 2010 ACR/EULAR classification criteria for RA[21], the 2019 EULAR/ACR classification criteria for SLE [22], and the ASAS classification criteria for SpA [23].

All participants were invited to participate and provided written informed consent. For participants younger than 18 years, written informed assent was obtained and consent was provided by a parent or legal guardian. The study was approved by the Research Ethics Committee of the Federal University of Uberlândia (CAAE 63571422.1.0000.5152 and 63623822.2.0000.5152).

Control Recruitment and Selection

Control participants were recruited from different locations in the municipality of Uberlândia, Minas Gerais, Brazil, including schools, educational centers, and residential neighborhoods. Controls were selected at enrollment to achieve close comparability with cases with respect to age, sex, and household income. Eligibility criteria for the control group included self-reported absence of chronic diseases and no history of continuous medication use.

Data Collection, Socioeconomic and Clinical Assessment

Socioeconomic and environmental information was collected using a structured questionnaire,

including household income, pet ownership, history of helminth infection, recent anthelmintic use, and neighborhood characteristics. To refine socioeconomic characterization, neighborhoods were classified according to the average land price per square meter, as previously validated by Lacerda (2024) [24]. Clinical and laboratory data, as well as current pharmacological treatment, were obtained from medical records. In patients with SLE, disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [25]. Functional disability was assessed using the Health Assessment Questionnaire-Disability Index (HAQ-DI) [26] in the groups for which these data were available. Peripheral blood eosinophil counts were retrieved from medical records corresponding to the date of blood collection.

Antigen Production and Purification

To combine broad serological screening with improved specificity, a sequential antigen strategy was adopted. Native *Toxocara canis* excretory-secretory (TES) antigen was used for initial screening because it represents the conventional serological antigenic preparation for human toxocariasis and contains a broad repertoire of larval secreted antigens. TES-reactive samples were then further evaluated using the recombinant chimeric antigen rSHORT to improve specificity and reduce cross-reactivity. Native *Toxocara canis* excretory-secretory (TES) antigens were produced *in vitro* as previously described by Thomas et al. (2016) [27]. Briefly, adult female worms were recovered from naturally infected dogs, and third-stage larvae (L3) were maintained in culture for TES collection. All samples were initially screened for anti-TES IgG. Samples reactive in this screening step were subsequently evaluated using the recombinant chimeric antigen rSHORT for confirmatory IgG testing and for determination of IgG1 and IgG4 seroreactivity.

The recombinant chimeric antigen rSHORT (Brazilian patent application No. BR 10 2024 011739-5 A2, filed with the Instituto Nacional da Propriedade Industrial — INPI) was developed by the Laboratory of Allergy and Acarology, Federal University of Bahia (UFBA), and described by Silva (2024) [20,28]. The construct was designed by combining linear B-cell epitopes derived from the *T. canis* TES-26 and CTL-4 proteins, cloned into the pET-21a(+) vector, and expressed in *Escherichia coli* BL21 (DE3). Previous validation studies indicated high diagnostic performance and reduced cross-reactivity with other helminths, supporting its use as a confirmatory antigen in the present study [20,28].

Immunological Assays

Peripheral blood samples (5 mL) were collected from all participants in tubes without anticoagulant. After centrifugation at 3000 rpm for 10 min, serum aliquots were obtained and stored at −80 °C until use.

Sequential serological strategy: a sequential antigen-based approach was used. All samples were initially screened for anti-*Toxocara* IgG using native TES antigen. TES-reactive samples were subsequently tested with the recombinant chimeric antigen rSHORT for confirmatory IgG assessment and for determination of IgG1 and IgG4 seroreactivity. Importantly, IgG1 and IgG4 assays were performed in TES-reactive samples, rather than being restricted only to samples additionally confirmed as rSHORT-IgG-positive.

Indirect ELISA was performed using 96-well microplates (Corning Costar®, Ref. 3590). Wells were coated overnight at 4 °C with 100 µL/well of antigen at optimized concentrations: 3 µg/mL for native TES and 4.5 µg/mL for rSHORT, diluted in 0.05 M carbonate-bicarbonate buffer (pH 9.6). After washing three times with PBS containing 0.05% Tween-20 (PBS-T), non-specific binding sites were blocked with 5% skim milk (Molico, Nestlé) in PBS-T for 1 h at 37 °C. Serum samples were diluted 1:100 in blocking buffer and incubated for 1 h at 37 °C in duplicate.

For total IgG detection, plates were incubated with peroxidase-conjugated goat anti-human IgG (Fc specific) (Sigma-Aldrich/Merck, Cat. A0170) diluted 1:10,000. For subclass determination, plates were incubated with peroxidase-conjugated mouse monoclonal anti-human IgG1 (clone 8c/6-39, Cat. SAB4200768) diluted 1:4,000, or peroxidase-conjugated mouse monoclonal anti-human IgG4 (clone HP-6025, Cat. SAB4200770) diluted 1:1,000. Reactions were developed with o-phenylenediamine dihydrochloride (OPD) substrate (SIGMAFAST™, P9187, Sigma-Aldrich) for 15 min in the dark and stopped with 50 µL of 2 N H_2SO_4. Absorbance was measured at 490 nm using a microplate reader (BioTek). The cut-off value was defined as the mean optical density (OD) of negative controls plus three standard deviations. Results were expressed as reactivity index (RI; sample OD/cut-off OD); samples with RI ≥ 1.0 were considered positive.

Serum IL-10 and IL-17 levels were measured using DuoSet® sandwich ELISA kits (R&D Systems; DY217B and DY317, respectively), according to the manufacturer's instructions. The lowest non-zero standard concentration of the calibration curve was adopted as the lower reporting limit, corresponding to 31.3 pg/mL for IL-10 and 15.6 pg/mL for IL-17. Values below these thresholds were recorded as 0 for descriptive analyses.

Statistical Analysis

Statistical analyses were performed using Python (version 3.10) with the pandas, scipy, statsmodels, and scikit-learn libraries. Continuous variables were compared between groups using the Mann–Whitney U test, whereas categorical variables were compared using Fisher's exact test. Seroreactivity frequencies were calculated using the total number of individuals in each study group as the denominator. Multivariable logistic regression models were used to estimate odds ratios (ORs) for seroreactivity according to disease status, with adjustment for age, sex, and household income. Associations between immune markers and clinical outcomes were evaluated using ordinary least squares regression with HC3 robust standard errors for HAQ-DI and negative binomial regression for SLEDAI. For descriptive cytokine analyses, values below the lower reporting limits were recorded as 0. All tests were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

Demographic , socioeconomic, and baseline immunological characteristics

The study included 442 individuals distributed across three disease-specific case-control comparisons. As shown in Table 1, age, sex, and household income did not differ significantly between patients and their respective controls in any pair (all $p > 0.05$). History of helminth infection and anthelmintic use in the previous 6 months also did not differ significantly between cases and controls in any of the three pairs (all $p > 0.05$).

Differences in other baseline variables were pair-specific. In the RA pair, the neighborhood m² value category differed between cases and controls (2.00 [2.00–4.00] vs 4.00 [3.00–5.00], $p < 0.001$). In the SpA pair, pet ownership was more frequent among patients than among controls (82.5% vs 50.0%, $p < 0.001$). No significant pairwise differences were detected for these variables in SLE vs CT-SLE.

Among cytokine-related variables, IL-10 did not differ significantly between RA and CT-RA or between SLE and CT-SLE, but SpA showed lower IL-10 than CT-SpA, both for median values ($p = 0.040$) and for the proportion of detectable samples ($p = 0.043$). IL-17 measurements were available only for patient groups with comparable data and therefore were not directly compared with controls. Eosinophil counts were available only for case groups and are presented descriptively in Table 1.

Selective reduction of IgG4 seroreactivity in SLE

Serological analysis based on total IgG showed that anti-*Toxocara* seroreactivity was frequent across the study population (Fig. 1). In adjusted logistic regression models, only SLE showed significantly higher odds of IgG seroreactivity than its control group (OR 2.44, 95% CI 1.16–5.14; p = 0.019) (Table 2). RA and SpA showed higher point estimates than their respective controls, but these differences did not reach statistical significance (RA: OR 1.90, p = 0.056; SpA: OR 2.26, p = 0.054) (Table 2). IgG1 seroreactivity did not differ significantly between cases and controls in any of the three disease-control pairs (Table 2).

In contrast, IgG4 seroreactivity differed selectively in SLE. Whereas RA and SpA did not differ significantly from their controls for IgG4 seroreactivity, SLE showed lower odds of IgG4 seroreactivity than CT-SLE (OR 0.20, 95% CI 0.04–0.99; p = 0.049) (Table 2). Thus, in the SLE pair, increased total IgG seroreactivity coexisted with reduced IgG4 seroreactivity.

Clinical correlates of immune markers

Associations between immune markers and clinical outcomes were evaluated in disease-specific models. In RA and SpA, no independent association was detected between seroreactivity markers and HAQ-DI after adjustment. In both groups, household income was inversely associated with HAQ-DI (Online Resource 1).

In SLE, disease activity was assessed with a negative binomial regression model. Higher eosinophil counts were independently associated with lower SLEDAI scores (β = -0.006, p = 0.008) (Fig. 2). IgG4 seroreactivity was not independently associated with SLEDAI. No detectable differences in glucocorticoid, DMARD, or biologic use were observed according to IgG4 serostatus in SLE; however, this comparison was limited by the extremely small number of IgG4-reactive individuals.

Discussion

Our findings identify a distinctive serological pattern in systemic lupus erythematosus (SLE), characterized by preserved anti-*Toxocara* total IgG seroreactivity but selective reduction of IgG4. Because the IgG4 result was close to the conventional significance threshold and involved few IgG4-reactive individuals, this finding should be interpreted as a cautious, hypothesis-generating signal rather than definitive evidence of mechanistic IgG4 suppression. In contrast, rheumatoid arthritis (RA) and spondyloarthritis (SpA) did not differ significantly from their controls with respect to IgG4 seroreactivity. The use of the recombinant chimeric

antigen rSHORT, designed to improve specificity and reduce cross-reactivity relative to native antigens, strengthens the interpretation that the reduced IgG4 signal observed in SLE reflects a selective alteration in the quality of the anti-*Toxocara* humoral response rather than a nonspecific serological artifact [8,20].

This interpretation should be considered in light of the sequential serological strategy adopted in the present study. All samples were initially screened with native TES-IgG, whereas TES-reactive samples were subsequently evaluated with rSHORT for confirmatory IgG and subclass determination. Accordingly, the apparent coexistence of preserved total IgG seroreactivity and reduced IgG4 in SLE does not represent an internal inconsistency, but rather suggests that broad serological reactivity to *Toxocara* antigens may be maintained while the more specific regulatory-associated IgG4 response is selectively diminished.

This pattern is noteworthy because SLE is classically associated with B-cell hyperactivity and broad antibody production [19]. In that context, the reduction of IgG4 is not consistent with a generalized inability to mount humoral responses. Instead, the findings raise the possibility of a qualitative distortion of the response. In chronic human helminth infections, IgG4 has been linked to more regulated immune profiles [15], and helminths more broadly are recognized as potent inducers of immunoregulatory pathways, including those involving IL-10 and regulatory T cells [1,14]. Experimental and review data in lupus also suggest that helminth-derived immunomodulation may attenuate disease through regulatory mechanisms [16]. Taken together, our data are compatible with the possibility that the regulatory-associated humoral component of anti-*Toxocara* immunity may be altered in SLE, even when overall anti-*Toxocara* seroreactivity is preserved.

At the clinical level, the most robust disease-specific correlate identified in the present study was eosinophilia in SLE. Higher eosinophil counts were independently associated with lower SLEDAI scores, whereas IgG4 seroreactivity was not independently associated with disease activity in the intragroup model. This distinction is important. The between-group comparison indicates that IgG4 is reduced in SLE relative to controls, but the within-group clinical correlate detectable in SLE was eosinophils rather than IgG4 itself. Experimental toxocariasis is well known to induce eosinophil-rich type 2 inflammation that may be granulomagenic and pathogenic in some settings [12,13]. However, our findings suggest that, within SLE, eosinophilia may also mark a residual type 2/regulatory component associated with lower disease activity. This interpretation is in line with recent clinical observations linking peripheral eosinophil counts to disease activity and outcomes in lupus nephritis [29].

The results in RA and SpA should be interpreted more cautiously. For total IgG seroreactivity,

both RA and SpA showed higher adjusted point estimates than their corresponding control groups, with p values close to the conventional threshold for statistical significance. These directional patterns suggest that increased anti-*Toxocara* IgG seroreactivity may not be exclusive to SLE, although the statistical evidence was strongest in SLE. Helminth-derived molecules can attenuate inflammatory pathways in experimental arthritis models [18], and we therefore considered the possibility that serological markers of anti-*Toxocara* immunity might also correlate with better functional status in human autoimmune rheumatic diseases. In RA and SpA, no independent association was detected between IgG, IgG1, or IgG4 seroreactivity and HAQ-DI after adjustment. In both groups, household income remained inversely associated with HAQ-DI, whereas the serological markers did not. These findings suggest that the relationship between helminth-related humoral markers and clinical expression is likely to be disease-specific rather than uniform across autoimmune rheumatic diseases.

The cytokine findings also require cautious interpretation. Pairwise differences in IL-10 were not observed for SLE versus controls, whereas lower IL-10 levels and lower IL-10 detectability were observed in SpA relative to CT-SpA. Because serum IL-10 values were strongly concentrated at the lower end of the assay range, these findings should be interpreted conservatively. More broadly, although SLE has often been discussed in the context of Th17/Treg disequilibrium and impaired regulatory pathways [30,31], our serum cytokine results do not support a simple model in which SLE is uniquely defined by lower circulating IL-10 than its controls. Rather, they suggest that serum IL-10 may not be a sufficiently stable standalone surrogate of regulatory status in this setting.

From an epidemiological perspective, the recruitment strategy based on comparability between cases and controls strengthens the interpretation of the serological findings. Patients and controls were balanced by age, sex, and household income, and no significant pairwise differences were observed for reported history of helminth infection or recent anthelmintic use. This reduces the likelihood that the main serological differences were driven primarily by major demographic or socioeconomic imbalance. Notably, the disease-specific baseline differences observed in neighborhood m² value category in RA and pet ownership in SpA did not parallel the main serological pattern, which was most clearly supported in SLE. Although pet ownership is epidemiologically relevant to *Toxocara* exposure, its higher frequency in SpA was not accompanied by a statistically significant adjusted serological association, indicating that this variable alone is unlikely to explain the main findings. At the same time, the persistence of frequent anti-*Toxocara* seroreactivity across groups is consistent with sustained exposure in this setting, which is compatible with the known epidemiology of

toxocariasis in Brazil and with the public health relevance of canine and feline environmental contamination in urban areas [6,9–11,32].

The possible influence of treatment must also be interpreted with caution. No detectable differences in glucocorticoid, DMARD, or biologic use were observed according to IgG4 serostatus in SLE; however, the number of IgG4-positive individuals in this group was extremely small. Accordingly, these analyses cannot be taken as definitive evidence that treatment plays no role, but they also do not provide detectable support for the idea that the reduced IgG4 frequency in SLE was simply a consequence of immunosuppressive therapy.

These findings should be interpreted in light of several methodological considerations. The cross-sectional design does not allow causal inference regarding helminth exposure and autoimmune disease trajectories. In addition, subclass analyses were restricted to TES-reactive samples, which should be considered when interpreting the relationship between total IgG and subclass frequencies. Serology also cannot distinguish active infection from past exposure, even when a more specific recombinant antigen is used. Some subgroup analyses were constrained by small numbers, particularly the very low frequency of IgG4-reactive individuals in SLE. Accordingly, statistically borderline findings, especially those involving IgG4 in SLE and total IgG in RA and SpA, should be interpreted as hypothesis-generating and require confirmation in larger cohorts. These limitations should be balanced against important strengths, including the case-control design, the use of a recombinant chimeric antigen with improved specificity, and the integration of serological, cytokine, eosinophil, and clinical data across three autoimmune rheumatic disease groups.

In summary, the data support a model in which autoimmune rheumatic diseases do not share a uniform anti-*Toxocara* immune profile. The most distinctive pattern was observed in SLE, where preserved total IgG seroreactivity coexisted with selective reduction of IgG4, and where eosinophils emerged as the main independent correlate of lower disease activity. These findings support the view that the quality, rather than merely the presence, of anti-helminth immunity may be altered in autoimmune disease, particularly in SLE.

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Statements & Declarations

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Author Contributions

L.C.R., N.B.P., and R.R.C.-M. conceptualized and designed the study. L.C.R., V.C.M., T.S.P., I.A.F., L.V.O.L., B.M.S., and M.L.A. conducted participant interviews, collected biological samples, performed ELISA assays, and contributed to data analysis and manuscript revision. H.M.R. and R.R. provided clinical resources and patient diagnosis and contributed to the analysis and interpretation of clinical data and manuscript revision. R.C.S. and C.S.P. developed and provided the recombinant antigens and contributed to data analysis, interpretation of results, and manuscript revision. L.F.C.A. provided TES antigen material and contributed to data analysis, interpretation of results, and manuscript revision. C.G.M. and R.R.C.-M. performed the formal statistical analysis. L.C.R. wrote the original draft of the manuscript. R.R.C.-M. supervised the project. N.B.P. and R.R.C.-M. acquired funding. All authors critically revised the manuscript, approved the final version, and agree to be accountable for all aspects of the work.

Data availability

The de-identified data underlying this study are not publicly available because they contain sensitive information from human participants. Data and analysis scripts are available from the corresponding author upon reasonable request, subject to ethical and institutional approval where applicable.

Declarations

Ethics approval

This study was approved by the Research Ethics Committee of the Federal University of Uberlândia (CAAE 63571422.1.0000.5152 and 63623822.2.0000.5152).

Consent to participate

All participants provided written informed consent. For participants younger than 18 years, written informed assent was obtained and consent was provided by a parent or legal guardian.

Consent for publication

Not applicable.

Competing interests

Raphael C. Silva and Carina S. Pinheiro are listed as inventors/co-inventors on Brazilian

patent application No. BR 10 2024 011739-5 A2, filed with the Instituto Nacional da Propriedade Industrial (INPI), related to recombinant chimeric antigens for toxocariasis immunodiagnosis. The remaining authors declare no competing interests.

Table 1. Demographic, socioeconomic, clinical, cytokine, and serological characteristics of patients with autoimmune rheumatic diseases and their corresponding control groups

Variable	RA (n=89)	CT-RA (n=89)	SLE (n=75)	CT-SLE (n=76)	SpA (n=57)	CT-SpA (n=56)	p-value (RA vs CT-RA)	p-value (SLE vs CT-SLE)	p-value (SpA vs CT-SpA)
Demographic characteristics									
Age (years), median [IQR]	51.00 [41.00–59.00]	47.00 [40.00–55.00]	38.00 [28.50–43.50]	39.00 [32.00–47.00]	50.00 [41.00–58.00]	45.50 [31.25–55.25]	0.152	0.231	0.102
Female sex, n (%)	81 (91.0%)	76 (85.4%)	71 (94.7%)	72 (94.7%)	18 (31.6%)	18 (32.1%)	0.353	1.000	1.000
Socioeconomic and environmental variables									
Household income (minimum wages), median [IQR]	2.00 [1.00–3.00]	2.00 [1.00–3.50]	2.00 [1.50–3.00]	2.00 [1.38–3.00]	2.00 [1.50–2.50]	2.00 [1.00–4.12]	0.110	0.666	0.548
Neighborhood m² value category, median [IQR]	2.00 [2.00–4.00]	4.00 [3.00–5.00]	4.00 [2.25–4.00]	4.00 [2.00–5.00]	3.00 [2.00–4.50]	3.00 [2.00–4.00]	<0.001	0.245	0.596
Pet ownership (dog/cat), n (%)	57 (64.0%)	63 (70.8%)	56 (74.7%)	55 (72.4%)	47 (82.5%)	28 (50.0%)	0.424	0.854	<0.001
History of helminth infection, n (%)	38 (42.7%)	30 (33.7%)	28 (37.3%)	33 (43.4%)	23 (40.4%)	16 (28.6%)	0.280	0.508	0.236
Anthelmintic use in the last 6 months, n (%)	11 (12.4%)	16 (18.0%)	24 (32.0%)	17 (22.4%)	8 (14.0%)	8 (14.3%)	0.404	0.204	1.000
Cytokines and eosinophils									
IL-10 (pg/mL), median [IQR]	0.00 [0.00–0.00]	0.00 [0.00–0.00]	0.00 [0.00–0.00]	0.00 [0.00–0.00]	0.00 [0.00–0.00]	0.00 [0.00–0.00]	0.061	0.300	0.040
IL-10 detectable, n (%)	10 (11.2%)	18 (20.2%)	3 (4.0%)	1 (1.3%)	3 (5.3%)	10 (17.9%)	0.149	0.367	0.043
IL-17 (pg/mL), median [IQR]	0.00 [0.00–0.00]	—	0.00 [0.00–0.00]	—	0.00 [0.00–0.00]	—	—	—	—
IL-17 detectable, n (% of tested)	6 (6.7%)	—	6 (8.0%)	—	1 (1.8%)	—	—	—	—
Eosinophil count (/mm³), median [IQR]	161.00 [82.00–249.00]	—	78.00 [33.00–127.00]	—	161.50 [111.25–258.00]	—	—	—	—
Clinical indices									
HAQ-DI, median [IQR]	1.00 [0.38–	—	—	—	1.00 [0.25–	—	—	—	—

	1.62]				1.38]				
SLEDAI score, median [IQR]	—	—	0.00 [0.00–2.00]	—	—	—	—	—	—
Serological reactivity to rSHORT antigen									
IgG reactive, n (%)	33 (37.1%)	22 (24.7%)	28 (37.3%)	16 (21.1%)	26 (45.6%)	18 (32.1%)	0.104	0.032	0.178
IgG1 reactive, n (%)	10 (11.2%)	11 (12.4%)	5 (6.7%)	11 (14.5%)	11 (19.3%)	7 (12.5%)	1.000	0.185	0.442
IgG4 reactive, n (%)	10 (11.2%)	7 (7.9%)	2 (2.7%)	9 (11.8%)	9 (15.8%)	4 (7.1%)	0.611	0.056	0.238

Notes: Data are presented as median [IQR] for continuous variables and n (%) for categorical variables, unless otherwise indicated. P values compare each disease group with its respective control group. Percentages were calculated using the fixed total sample size of each group. — indicates not available or not assessed in a comparable manner. IL-10 and IL-17 values below the lower limit of detection were recorded as 0 for descriptive analysis. IL-17 was measured only in the patient groups with available comparable data; therefore, control groups are shown as —. For serology, all participants were initially screened by TES-IgG, and TES-reactive samples were subsequently tested for rSHORT-IgG, rSHORT-IgG1, and rSHORT-IgG4; however, the frequencies of IgG, IgG1, and IgG4 reactivity are expressed in relation to the total number of individuals in each group. P values were obtained using Mann–Whitney U tests for continuous variables and Fisher's exact tests for categorical variables. Abbreviations: RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SpA, spondyloarthritis; CT, control group; HAQ-DI, Health Assessment Questionnaire-Disability Index; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

Table 2. Adjusted associations between anti-*Toxocara* seroreactivity and autoimmune disease status

Comparison	Marker	Case reactive, n/N (%)	Control reactive, n/N (%)	Adjusted OR (95% CI)	P value	N included
RA vs CT-RA	IgG	33/89 (37.1%)	22/89 (24.7%)	1.90 (0.98–3.68)	0.056	178
	IgG1	10/89 (11.2%)	11/89 (12.4%)	0.82 (0.33–2.08)	0.683	178
	IgG4	10/89 (11.2%)	7/89 (7.9%)	1.71 (0.59–4.91)	0.322	178
SLE vs CT-SLE	IgG	28/75 (37.3%)	16/76 (21.1%)	2.44 (1.16–5.14)	0.019*	151
	IgG1	5/75 (6.7%)	11/76 (14.5%)	0.40 (0.13–1.24)	0.112	151
	IgG4	2/75 (2.7%)	9/76 (11.8%)	0.20 (0.04–0.99)	0.049*	151
SpA vs CT-SpA	IgG	26/57 (45.6%)	18/56 (32.1%)	2.26 (0.99–5.19)	0.054	113
	IgG1	11/57 (19.3%)	7/56 (12.5%)	1.46 (0.49–4.31)	0.493	113
	IgG4	9/57 (15.8%)	4/56 (7.1%)	3.30 (0.88–12.42)	0.078	113

Notes: Multivariable logistic regression models comparing rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and spondyloarthritis (SpA) groups with their respective control groups (CT-RA, CT-SLE, and CT-SpA). Models were adjusted for age, sex, and household income. Seroreactivity frequencies are presented as n/N (%) for each case and control group. Adjusted odds ratios (ORs) with 95% confidence intervals (95% CI) are shown for IgG, IgG1, and IgG4 seroreactivity. Asterisks indicate statistical significance at $p < 0.05$.

Figure legends

Fig. 1 Seroreactivity rates for anti-*Toxocara* antibodies in autoimmune rheumatic diseases and corresponding controls.

Legend: Bar plots showing the frequencies of anti-*Toxocara* seroreactivity for IgG, IgG1, and IgG4 in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and spondyloarthritis (SpA), compared with their control groups (CT-RA, CT-SLE, and CT-SpA). All participants were initially screened by TES-IgG, and TES-reactive samples were subsequently tested for rSHORT-IgG, rSHORT-IgG1, and rSHORT-IgG4. Seroreactivity frequencies are expressed as the percentage of the total number of individuals in each group. Brackets indicate statistically significant comparisons from multivariable logistic regression models adjusted for age, sex, and household income, and display the corresponding adjusted odds ratios (ORs), 95% confidence intervals (95% CIs), and p values. Significant differences were observed for SLE versus CT-SLE in total IgG and IgG4.

Fig. 2 Forest plot of adjusted predictors of disease activity in systemic lupus erythematosus.

Legend: Forest plot showing incidence rate ratios (IRRs) and 95% confidence intervals (95% CIs) from a negative binomial regression model evaluating predictors of disease activity, measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), in patients with systemic lupus erythematosus (SLE). The model included IgG4 seroreactivity, eosinophil count (expressed per 100 cells/mm^3), household income, age, and sex. The vertical dashed line at IRR = 1.0 indicates no association with SLEDAI. IRRs below 1.0 indicate association with lower expected disease activity, whereas IRRs above 1.0 indicate association with higher expected disease activity. Among the evaluated predictors, only eosinophil count was independently associated with lower SLEDAI scores.

Fig. 1

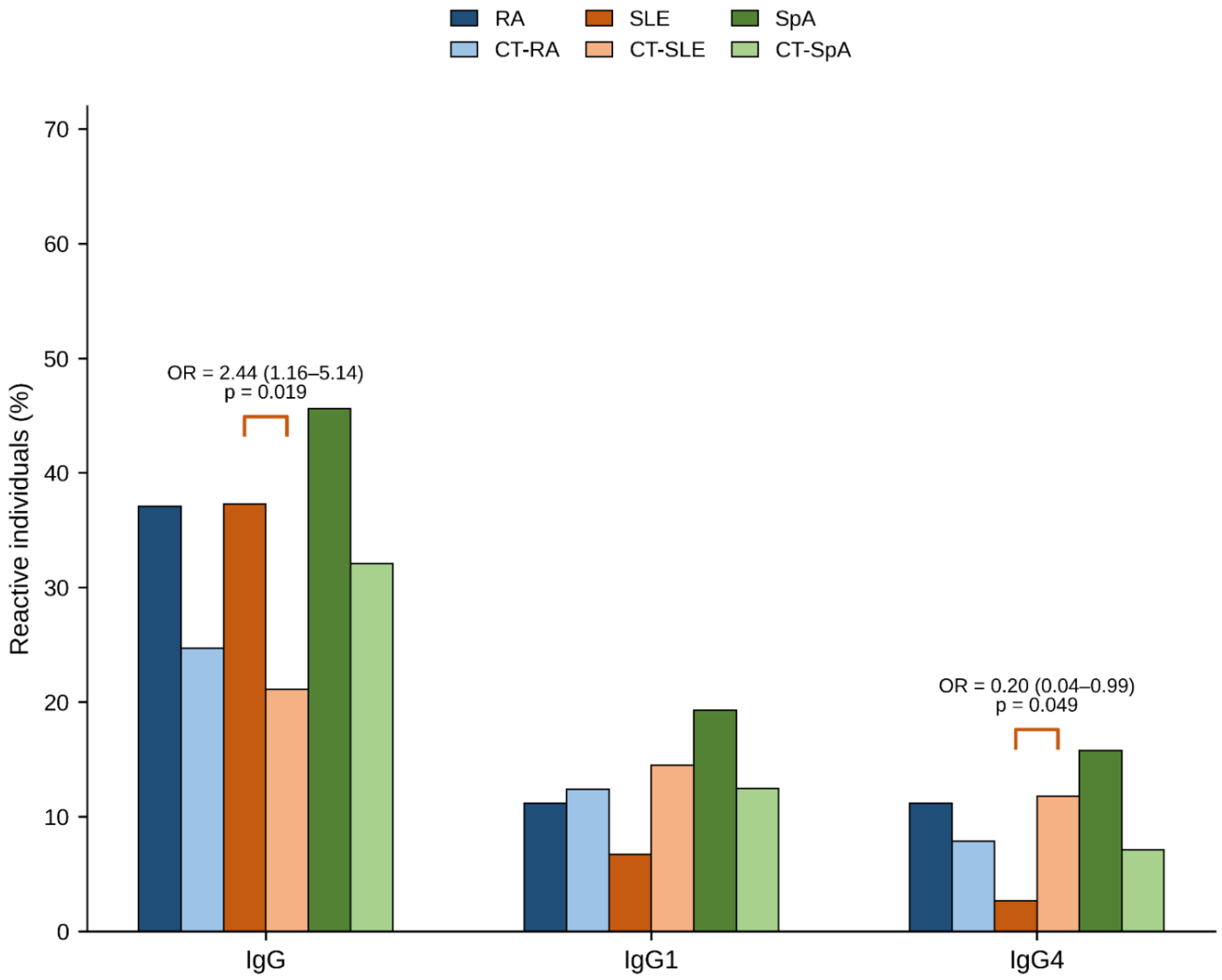
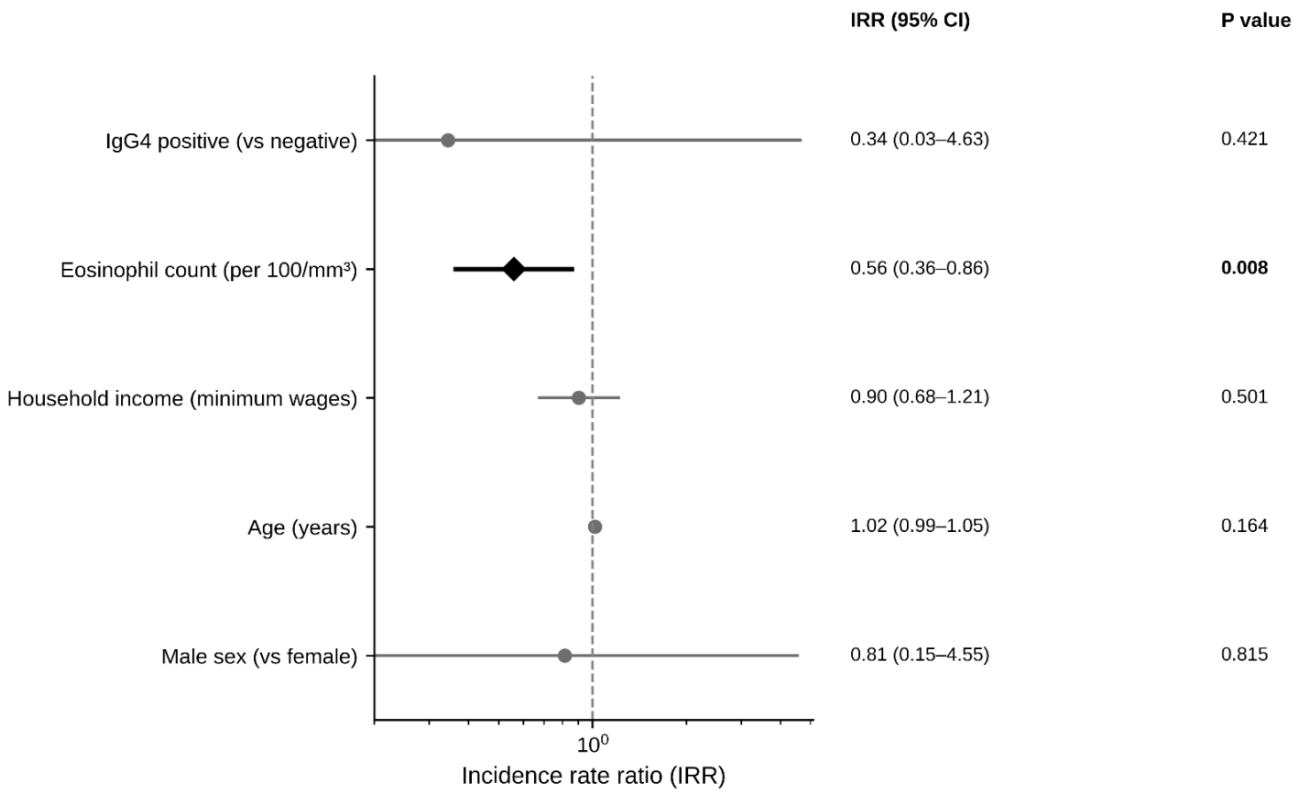

RA
SLE
SpA
CT-RA
CT-SLE
CT-SpA
Reactive individuals (%)
70
60
50
40
30
20
10
0
OR = 2.44 (1.16–5.14)
p = 0.019
OR = 0.20 (0.04–0.99)
p = 0.049
IgG
IgG1
IgG4

Fig. 2


IRR (95% CI)
P value
IgG4 positive (vs negative)
0.34 (0.03–4.63)
0.421
Eosinophil count (per 100/mm³)
0.56 (0.36–0.86)
0.008
Household income (minimum wages)
0.90 (0.68–1.21)
0.501
Age (years)
1.02 (0.99–1.05)
0.164
Male sex (vs female)
0.81 (0.15–4.55)
0.815
10⁰
Incidence rate ratio (IRR)

Online Resource 1. Multivariable linear regression models evaluating predictors of functional disability (HAQ-DI) in patients with rheumatoid arthritis and spondyloarthritis

Group	Variable	N included	β (95% CI)	P value	R^2
RA	Intercept	85	1.361 (0.507 – 2.216)	0.002	0.216
RA	IgG4 reactive (vs negative)	85	-0.262 (-0.740 – 0.216)	0.282	0.216
RA	Eosinophil count (/mm³)	85	-0.001 (-0.001 – 0.000)	0.168	0.216
RA	Household income (minimum wages)	85	-0.217 (-0.325 – -0.109)	<0.001	0.216
RA	Age (years)	85	0.007 (-0.007 – 0.021)	0.347	0.216
RA	Male sex (vs female)	85	-0.139 (-0.779 – 0.501)	0.670	0.216
SpA	Intercept	48	1.552 (0.589 – 2.516)	0.002	0.275
SpA	IgG4 reactive (vs negative)	48	-0.351 (-0.997 – 0.295)	0.287	0.275
SpA	Eosinophil count (/mm³)	48	0.000 (-0.001 – 0.002)	0.429	0.275
SpA	Household income (minimum wages)	48	-0.254 (-0.379 – -0.130)	<0.001	0.275
SpA	Age (years)	48	0.001 (-0.014 – 0.017)	0.871	0.275
SpA	Male sex (vs female)	48	-0.133 (-0.491 – 0.224)	0.465	0.275

Notes: Ordinary least squares linear regression models with HC3 robust standard errors evaluating predictors of functional disability, measured by the Health Assessment Questionnaire-Disability Index (HAQ-DI), in patients with rheumatoid arthritis (RA) and spondyloarthritis (SpA). Models included IgG4 seroreactivity, eosinophil count, household income, age, and sex. Regression coefficients (β), 95% confidence intervals (95% CIs), p values, and model R^2 are shown. Negative coefficients indicate association with lower HAQ-DI scores.

CONSIDERAÇÕES FINAIS

Os resultados desta dissertação demonstram que pacientes com doenças reumáticas autoimunes apresentam perfis heterogêneos de resposta sorológica anti-*Toxocara canis*, evidenciando diferenças importantes entre as enfermidades avaliadas. Entre elas, o lúpus eritematoso sistêmico destacou-se por apresentar um padrão imunológico distinto, caracterizado pela manutenção da sororreatividade para IgG total concomitante à redução seletiva da resposta IgG4 anti-*T. canis*.

Esse achado sugere que, embora pacientes com LES mantenham capacidade de desenvolver resposta humoral contra antígenos helmínticos, componentes associados à imunorregulação, particularmente a IgG4, podem estar qualitativamente alterados. Considerando o papel da IgG4 em contextos de resposta Th2 modificada e tolerância imunológica, os dados obtidos são compatíveis com a hipótese de que alterações qualitativas da resposta anti-helmíntica possam estar envolvidas na desregulação imunológica observada no LES.

Além disso, a associação independente entre maiores contagens de eosinófilos e menores índices de atividade da doença sugere que eosinófilos possam representar um marcador mais consistente de um eixo imunorregulador residual em pacientes lúpicos do que a própria IgG4. Esses resultados ampliam a compreensão sobre a complexa interação entre helmintos e autoimunidade, especialmente no contexto da hipótese dos “velhos amigos” e dos mecanismos imunomoduladores induzidos por parasitos.

A utilização da proteína recombinante quimérica rSHORT constituiu um diferencial metodológico importante do estudo, permitindo maior especificidade na avaliação sorológica e reduzindo potenciais reações cruzadas associadas ao uso exclusivo de antígenos nativos. Dessa forma, os achados obtidos contribuem não apenas para o entendimento da resposta imune anti-*Toxocara*, mas também para o avanço de estratégias diagnósticas mais precisas em estudos de helmintíases humanas.

Entre as limitações do estudo, destacam-se o delineamento transversal, que impossibilita estabelecer relações causais, e o fato de os marcadores sorológicos refletirem exposição e resposta imune, sem confirmação parasitológica direta de infecção ativa. Além disso, fatores relacionados ao uso de imunossupressores e à heterogeneidade clínica das doenças autoimunes podem influenciar os perfis imunológicos observados.

Por fim, os resultados apresentados reforçam a necessidade de novos estudos longitudinais e mecanísticos que investiguem de forma mais aprofundada os componentes regulatórios da resposta anti-helmíntica em doenças autoimunes, especialmente no LES. A compreensão dessas interações poderá contribuir para o desenvolvimento de biomarcadores imunológicos e, potencialmente, de novas abordagens terapêuticas baseadas em mecanismos de imunomodulação induzidos por helmintos.