UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

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O RECEPTOR P2X7 MODULA A MICROBIOTA INTESTINAL E O INFLAMASSOMA DETERMINANDO A GRAVIDADE DA ILEÍTE INDUZIDA PELO *TOXOPLASMA GONDII*

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Clínica Médica, Faculdade de Medicina da Universidade Federal do Rio de Janeiro, como requisito parcial à obtenção do título de Doutora em Ciências.

Orientadores: Prof. Dr. Heitor Siffert Pereira de Souza
Prof. Dr. Robson Coutinho Silva
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RESUMO

NANINI, H. F. **O receptor P2X7 modula a microbiota intestinal e o inflamassoma determinando a gravidade da ileíte induzida pelo *Toxoplasma gondii*.** Rio de Janeiro, 2023. Tese (Doutorado em Ciências) - Pós-Graduação em Clínica Médica, Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2023.

Em altas concentrações no meio extracelular, a molécula de ATP atua como um sinal de perigo capaz de modular a imunidade e a inflamação via a ativação de receptores P2X7. Este receptor é expresso por diferentes tipos celulares como macrófagos, linfócitos e células epiteliais intestinais. O receptor P2X7 é modulado positivamente por IFN-ɣ e a sua ativação induz apoptose e autofagia via produção de espécies reativas de oxigênio em células epiteliais intestinais. A ativação do receptor P2X7 representa um segundo sinal para a ativação do inflamassoma e induz a eliminação de parasitas intracelulares. A infecção oral por cistos de *Toxoplasma gondii* induz uma inflamação grave no íleo com características imunes e histológicas semelhantes as observadas na Doença de Crohn. O presente estudo tem como objetivo investigar o impacto do receptor P2X7 na ileíte induzida por *T. gondii*. A indução de ileíte foi realizada em camundongos WT e nocautes para o receptor P2X7 ($P2X7^{-/-}$) pela infecção oral com cistos de *T. gondii* da cepa ME49. Após 8 dias de infecção, os animais foram eutanasiados e amostras de sangue periférico e fragmentos do íleo foram coletados para diversas análises como dosagem de mediadores inflamatórios, histopatologia, imunohistoquímica, avaliação da contração ileal e expressão gênica por PCR em tempo real. Além disso, amostras de fezes foram coletadas para análise da microbiota fecal. A infecção oral por *T. gondii* induz o aumento da expressão do receptor P2X7 no íleo. A ileíte em camundongos $P2X7^{-/-}$ infectados apresenta-se mais severa, com aumento da mortalidade, carga parasitária, extensão do dano tecidual no íleo, dano hepático e aumento da contração intestinal em comparação com animais WT infectados. A concentração sérica de IL-6 e IFN-γ e a expressão tecidual de caspase-1 e NLRP3 não aumentaram em animais $P2X7^{-/-}$ infectados em comparação com os níveis mais elevados observados em camundongos WT. A análise da microbiota fecal demonstrou um aumento na população de *Bacteroidaceae*, *Rikenellaceae* e *Rhodospirillales* e redução em *Muribaculaceae* e *Lactobacillaceae* em animais WT e $P2X7^{-/-}$ infectados. Entretanto, *Bacteroidia* e *Tannerellaceae* apresentaram-se aumentadas em $P2X7^{-/-}$ enquanto *Clostridiales* e *Mollicutes* ausentes em $P2X7^{-/-}$ e aumentadas no grupo WT. Os resultados demostram que o receptor P2X7 protege os animas contra a infecção por *T. gondii*

pelo aumento da ativação do inflamassoma e controle da resposta inflamatória local e sistêmica. Além disso, populações específicas de bactérias intestinais moduladas pelo receptor P2X7 determinam a severidade da ileíte.

Palavras-chave: Ileíte, receptor P2X7, inflamassoma, *Toxoplasma gondii* e microbiota intestinal.

ABSTRACT

NANINI, H. F**. P2X7 receptor modulation of the gut microbiota and the inflammasome determines the severity of *Toxoplasma gondii*-induced ileitis.** Rio de Janeiro, 2023. Tese (Doutorado em Ciências) - Pós-Graduação em Clínica Médica, Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2023.

The ATP molecule in high concentrations in extracellular environment, act as a danger signal able to modulate immunity and inflammation by P2X7 receptor activation. This receptor is expressed by different cell types as macrophages, lymphocytes and intestinal epithelial cells. The P2X7 receptor is positively modulated by IFN-ɣ and its activation induces apoptosis and autophagy by production of reactive oxygen species in intestinal epithelial cells. The P2X7 receptor activation it present as a second signal to inflammasome activation and induces the elimination of intracellular parasites. The oral infection with *Toxoplasma gondii* cysts induces a severe inflammation in the ileum with immune and histological features similar to those seen in Crohn's disease. The objective of this study is to investigate the impact of the P2X7 receptor on *T. gondii*-induced ileitis. In this study, ileitis was induced in WT and knockout to P2X7 receptor ($P2X7^{-/-}$) mice by oral infection with *T. gondii* cysts of the ME49 strain. After 8 days of infection, the animals were euthanized and samples of peripheral blood and ileal fragments were collected for various analyzes such as measurement of inflammatory mediators, histopathology, immunohistochemistry, evaluation of ileal contraction and gene expression by real-time PCR. In addition, stool samples were collected for fecal microbiota analysis. The oral *T. gondii* infection induces increased P2X7 receptor expression in the ileum. Ileitis in infected $P2X7^{-/-}$ mice is more severe, with increased mortality, parasitic burden, extent of tissue damage in the ileum, liver damage and increased intestinal contraction compared to infected WT mice. Serum IL-6 and IFN-ɣ concentration and tissue expression of caspase-1 and NLRP3 did not increase in infected $P2X7^{-/-}$ mice compared to the higher levels observed in WT mice. Fecal microbiota analysis showed na increase in the population of *Bacteroidaceae, Rikenellaceae* e *Rhodospirillales* and a reduction in *Muribaculaceae* and *Lactobacillaceae* in infected WT and $P2X7^{-/-}$ mice. However, *Bacteroidia* and *Tannerellaceae* were increased in KO P2X7 while *Clostridiales* and *Mollicutes* were absent in $P2X7^{-/-}$ and increased in the WT group. The results demonstrate that the P2X7 receptor protects mice against *T. gondii* infection by increasing inflammasome activation and controlling the local and systemic inflammatory response. Furthermore,

specific population of gut bacteria modulated by the P2X7 receptor determine the severity of ileitis.

Keywords: Ileitis, P2X7 receptor, inflammasome, *Toxoplasma gondii* and gut microbiota.

LISTA DE ABREVIATURAS

ALT	Alanina aminotransferase
AST	Aspartato aminotransferase
ATP	Adenosina trifosfato
DAMP	Padrões moleculares associados ao dano
DC	Doença de Crohn
DII	Doença inflamatória intestinal
IFN-γ	Interferon gama
IL	Interleucina
iNOS	Óxido nítrico sintase induzida
NF-κB	Fator nuclear kappa B
NO	Óxido Nítrico
PAMP	Padrões moleculares associados a patógenos
PRRs	Receptores de reconhecimento de padrão
ROS	Espécies reativas de oxigênio
TLR	Receptores do tipo Toll-like
TNF-α	Fator de necrose tumoral alfa

SUMÁRIO

INTRODUÇÃO

O protozoário *Toxoplasma gondii* (*T. gondii*) pertence ao filo Apicomplexa e é o agente etiológico da toxoplasmose. Em muitos casos, *T. gondii* desencadeia doença assintomática, mas pode ser fatal em pacientes imunocomprometidos, por isso é considerado um parasita oportunista. A toxoplasmose é uma doença negligenciada que afeta 30% da população mundial (MOREIRA-SOUZA & COUTINHO-SILVA, 2021), e em algumas áreas geográficas, como o Brasil por exemplo, até 60% da população é soropositiva para antígenos de *T. gondii* (DUBEY *et al.*, 2012).

O *T. gondii* é um parasita intracelular obrigatório capaz de infectar todos os tipos de células nucleadas de mamíferos e aves. A reprodução sexuada do parasita ocorre no intestino dos membros da família Felidae, incluindo o gato doméstico, portanto são considerados hospedeiros definitivos. Nos hospedeiros intermediários, ocorre apenas a reprodução assexuada do parasita. Em seu ciclo de vida, o *T. gondii* apresenta três formas evolutivas capazes de infectar hospedeiros intermediários e definitivos denominadas taquizoítas, bradizoítas e esporozoítas, como pode ser observado na Figura 1 (ATTIAS *et al.*, 2020).

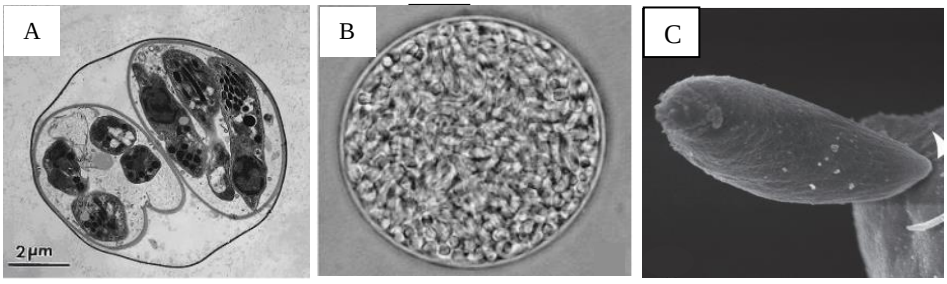

Figura 1. Formas biológicas do ciclo de vida do protozoário *Toxoplasma gondii*. **A.** Oocistos contendo esporozoítas. **B.** Cistos teciduais contendo bradizoítas. **C.** Taquizoítas. (Adaptado de DUBEY *et al*., 1998 e SOUZA & BELFORT, 2014).

Os taquizoítas invadem as células do hospedeiro por penetração ativa, e são encontrados no interior de vacúolos parasitóforos. Os taquizoítas se multiplicam por via assexuada e, após completarem o seu ciclo lítico, rompem a membrana plasmática da célula hospedeira. No meio extracelular, se disseminam para diferentes tecidos. Os taquizoítas se diferenciam em bradizoítas, forma biológica capaz de alterar a membrana e a matriz do vacúolo parasitóforo, levando a formação de uma parede cística e originando o cisto tecidual. Os oocistos contendo esporozoítas se formam através da reprodução sexuada do parasita no epitélio intestinal de gatos e outros felídeos, que são os hospedeiros definitivos do parasita.

Essa forma biológica é liberada para o meio ambiente junto com as fezes do animal infectado, como pode ser observado na Figura 2 (SOUZA & BELFORT, 2014).

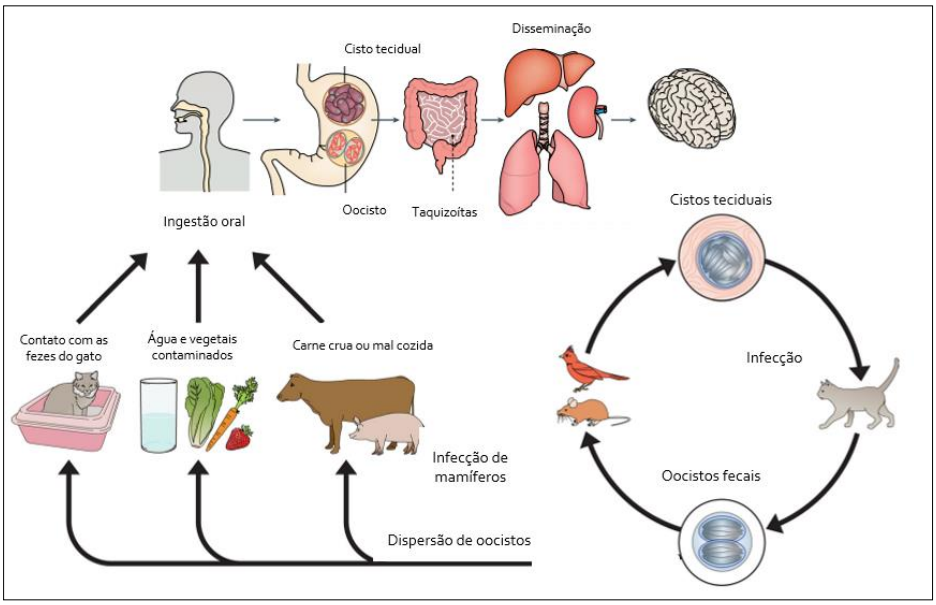

Figura 2. Ciclo de vida do protozoário *Toxoplasma gondii*. A replicação sexuada do protozoário ocorre no intestino de gatos e, por isso, esses animais são considerados os hospedeiros definitivos do parasita. A infecção desses animais acontece por carnivorismo, através da ingestão de cistos teciduais contendo bradizoítas ou pela ingestão de oocistos. Os parasitas infectam os enterócitos do hospedeiro definitivo e neles ocorre a replicação sexuada do parasita, onde gametas femininos e masculinos são formados por merogonia. Em seguida, os gametas se fundem, o que leva à formação de oocistos. Os oocistos do parasita são eliminados nas fezes de gatos e sobrevivem no ambiente por um longo período. Após sua maturação, os oocistos esporulados (contendo esporozoítas) podem contaminar alimentos e água, fornecendo uma rota de infecção para hospedeiros intermediários. Nos hospedeiros intermediários, ocorre a reprodução assexuada do parasita. Os seres humanos são infectados pela ingestão de carne crua ou malcozida contendo cistos teciduais ou pela ingestão de oocistos esporulados em água contaminada. Após a ingestão de cistos teciduais ou oocistos, o *T. gondii* infecta células do intestino delgado e estabelece a infecção aguda da doença, que é caracterizada pela presença de taquizoítas. Os taquizoítas se disseminam sistemicamente no hospedeiro e migram para sítios de infecção preferenciais, como sistema nervoso central, estabelecendo uma infecção crônica ao longo da vida do hospedeiro, através da formação de cistos (Modificado de MATTA *et al*., 2021 e ESCH & PETERSEN, 2013).

Os hospedeiros intermediários podem ser infectados pela ingestão de água, vegetais e frutas contaminadas com oocistos derivados das fezes de gatos, além de ingestão de carne crua ou malcozida contendo cistos teciduais (Figura 2). Ademais, a transmissão também pode ocorrer em humanos via transplante de órgãos, transfusão de sangue ou transmissão congênita. O hospedeiro definitivo pode se infectar através de carnivorismo ou ingestão de

oocistos (ATTIAS *et al.*, 2020). A suscetibilidade à toxoplasmose é determinada pela capacidade do sistema imunológico do hospedeiro de controlar a replicação do parasita (MOREIRA-SOUZA *et al*., 2019).

Após a ingestão oral de *T. gondii*, cistos e oocistos sofrem desencistamento após passagem pelo estômago e, em seguida, as formas biológicas infectantes do parasita são liberadas no intestino delgado. O *T. gondii* invade o intestino delgado do hospedeiro, infecta e se prolifera em células epiteliais intestinais. Enterócitos e células caliciformes são apontadas como os primeiros tipos de células epiteliais intestinais a serem infectadas pelo parasita (WILHELM & YAROVINSKY, 2014). O protozoário se diferencia em taquizoítas, constituindo a fase aguda da toxoplasmose, caracterizada pela maior taxa de replicação do *T. gondii* no intestino, linfonodos e órgãos viscerais (MOREIRA-SOUZA *et al*., 2019). A carga parasitária no intestino aumenta significativamente entre 3 e 7 dias após a infecção (WILHELM & YAROVINSKY, 2014).

As células epiteliais intestinais são sensíveis e respondem ao estímulo microbiano para reforçar a função da barreira e coordenar a resposta imune apropriada que dependem de mecanismos locais e sistêmicos (QUAN *et al*., 2018). Após a exposição ao parasita, as células epiteliais intestinais reconhecem o protozoário através de receptores de reconhecimento de padrão (PRRs) presentes na superfície celular, que induzem a liberação de citocinas pró-inflamatórias e contribuem a indução da resposta imunológica do tipo Th1 (DELGADO BETANCOURT *et al*., 2019). As células de Paneth, uma célula epitelial intestinal produtora de peptídeos antimicrobianos no intestino, diminuem em número durante a infecção por *T. gondii*. A perda desse tipo celular está associada ao aumento de IFN- γ desencadeado pela infecção (SNYDER & DENKERS, 2021).

A replicação do parasita leva à lise das células infectadas, culminando na liberação de padrões moleculares associados ao dano (DAMPs) que contribuem para a resposta inflamatória frente à infecção. Além disso, a rápida proliferação de *T. gondii* no epitélio intestinal leva ao rompimento da barreira e perda de sua integridade, fatores que permitem a translocação de componentes da microbiota para a lâmina própria que exacerbam a resposta inflamatória (SARDINHA-SILVA *et al*, 2022). Por isso, a composição da microbiota intestinal pode influenciar a patofisiologia da infecção parasitária e mudanças na microbiota podem conferir resistência ou favorecer a infecção (SHAO *et al*., 2020).

A infecção por *T. gondii* induz alterações na microbiota intestinal de camundongos, onde foi observada uma mudança significativa na abundância relativa de bactérias cecais, levando ao aumento de bactérias nocivas como Proteobacterias e a diminuição de bactérias

benéficas como Lactobacillus. Esses resultados sugerem que a microbiota intestinal pode conferir papel importante na progressão da toxoplasmose de infecção aguda para infecção crônica (SHAO *et al*., 2020). Além disso, observou-se que a ausência de bactérias comensais intestinais impediu o desenvolvimento de ileíte após infecção de camundongos selvagens com *T. gondii* (PARTIDA-RODRÍGUEZ *et al*., 2017).

A infecção por *T. gondii* induz a resposta imunológica do tipo Th1 no intestino caracterizada pela produção de IFN- γ e IL-12, sendo essencial para o controle do parasita. O IFN- γ é a principal citocina que participa no controle da infecção. Além de desencadear a produção de citocinas Th1, estimula a produção de óxido nítrico (NO), espécies reativas de oxigênio (ROS) e peptídeos antimicrobianos. Em adição, outras citocinas pró-inflamatórias contribuem na resposta imunológica contra o parasita, como TNF-α, IL-1β e IL-18 (PARTIDA-RODRÍGUEZ *et al*., 2017). Embora a resposta por células T CD4 do tipo Th1 predomine durante a infecção por *T. gondii*, as células do tipo Th17 também desempenham papel na imunidade celular contra o parasita (SNYDER & DENKERS, 2021). Além disso, a infecção por *T. gondii* induz o colapso de células T regulatórias intestinais (SARDINHA-SILVA *et al*, 2022).

Na lâmina própria, o *T. gondii* infecta e utiliza células como macrófagos e células dendríticas para migrar para sítios de infecção preferenciais, como o ambiente imune protegidos do sistema nervoso central, estabelecendo uma infecção crônica ao longo da vida do hospedeiro. O parasita sobrevive dentro das células hospedeiras superando muitos mecanismos antimicrobianos, incluindo a produção de ROS e NO, fusão lisossômica (para o vacúolo parasitóforo), indução da morte da célula hospedeira e a secreção de citocinas e quimiocinas pró-inflamatórias. Concomitantemente, o *T. gondii* desencadeia a ativação de fatores de transcrição anti-inflamatórios nas células hospedeiras, induzindo a superexpessão dos receptores envolvidos na migração da célula para o cérebro, que caracterizam a fase crônica da toxoplasmose (MOREIRA-SOUZA *et al*., 2017).

A infecção por *T. gondii* induz uma inflamação grave no intestino delgado. Em camundongos, essa patologia compartilha características imunológicas, morfológicas e histológicas com as observadas em doenças inflamatórias intestinais (DIIs), como indução sistêmica e local da resposta inflamatória, perda da arquitetura da barreira epitelial intestinal, encurtamento das vilosidades, fluxo maciço de células inflamatórias para a lâmina própria e sítios de necrose tecidual. Essa inflamação, quando desregulada, resulta na mortalidade precoce dos hospedeiros suscetíveis. Devido a infecção oral por cistos de *T. gondii* desencadear uma inflamação intestinal em camundongos com características inflamatórias

semelhantes às apresentadas na Doença de Crohn (DC), a toxoileíte é um modelo amplamente utilizado para o estudo dessa patologia (KASPER *et al*., 2004).

Em condições fisiológicas, os nucleotídeos como o ATP estão presentes em altas concentrações no meio intracelular (5 - 10 mM) e em concentrações extracelulares na faixa nanomolar (DI VIRGILIO, 2005). Após estimulação por diferentes estresses celulares como necrose, apoptose, hipóxia ou invasão de patógenos, as células podem liberar nucleotídeos para o meio extracelular. Nesse ambiente, o ATP atua como um sinal de perigo capaz de modular a imunidade e a inflamação via a ativação de receptores de membrana conhecidos como receptores P2 (NANINI *et al*., 2018).

A família de receptores P2 é subdividida nas subfamílias P2X e P2Y. Os receptores P2Y são acoplados à proteína G trimérica, são compostos por oito subtipos em mamíferos (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13 e P2Y14) e possuem sete domínios transmembrana, sendo o domínio aminoterminal voltado para o meio extracelular e o carboxiterminal para o meio citoplasmático. Os receptores P2X são canais iônicos ativados por ligantes, tendo sido descrito sete membros em mamíferos (P2X1-7). Esses receptores possuem suas porções aminoterminal e carboxiterminal voltadas para o meio intracelular (DI VIRGILIO *et al*., 2001; BURNSTOCK, 2007).

O receptor P2X7 difere dos outros membros da família P2X por possuir sua cauda carboxiterminal mais alongada, sendo essa associada à regulação de suas funções, ativação dos mecanismos de sinalização e localização celular. O receptor P2X7 é expresso por diversos tipos celulares como monócitos, macrófagos, linfócitos, neuronais, fibroblastos, endoteliais e epiteliais (BURNSTOCK & KNIGHT, 2004; CHEN *et al*., 2005; WELTER-STAHL *et al*. 2009).

A ativação de P2X7 induz a abertura de um poro permeável a moléculas de até 900 Da (BURNSTOCK, 2007) e o influxo de Na^+ e Ca^{2+} e o efluxo de K^+, resultando em mudanças na homeostase iônica da célula. Esse receptor pode iniciar a liberação em grande escala de ATP intracelular através de sua habilidade intrínseca de formação de poro ou em associação com hemicanais de panexina, aumentando a sinalização purinérgica e inflamação (SAVIO *et al*., 2018).

Diversas funções têm sido atribuídas ao receptor P2X7 na resposta imune inata e adaptativa. Durante a resposta imune inata, DAMPs ou PAMPs ativam receptores de reconhecimento de padrão (PRRs), por exemplo, receptores Toll-like (TLRs), induzindo a liberação de ATP, que pode ativar o receptor P2X7. Em adição, o mecanismo de ativação de NF-κB mediado por TLR age como um primeiro sinal promovendo a transcrição de diversos

genes codificadores de mediadores inflamatórios incluindo pró-IL-1β, pró-IL-18 e componentes do inflamassoma, como NLRP3 e ASC. A estimulação do receptor P2X7 representa um segundo sinal para a ativação do inflamassoma por desencadear o influxo de Ca^{2+} e o efluxo de K^{+}, a montagem do inflamassoma e subsequente ativação de caspase-1. Essa, por sua vez, processa pró-IL-1β em sua forma madura que é capaz de ser liberada (SAVIO *et al*., 2018). Adicionalmente, a ativação do receptor P2X7 induz a maturação e a liberação das citocinas pró-inflamatórias IL-8 e TNF-α (LISTER *et al*., 2007).

A ativação do receptor P2X7 também induz o recrutamento e a migração de neutrófilos (CESARO *et al*., 2010), ativação de diferentes fosfolipases (COUTINHO-SILVA *et al*., 2003; COSTA-JUNIOR *et al*., 2011), estimula a produção de radicais livres e participa na regulação do ciclo celular e indução de apoptose (NANINI *et al*., 2018). O receptor P2X7 é modulado positivamente por IFN-ɣ em células epiteliais intestinais (WELTER-STAHL *et al*., 2009) e a sua ativação induz apoptose e autofagia via produção de ROS (SOUZA *et al*., 2012). Além disso, a ativação do receptor P2X7 induz a eliminação de patógenos intracelulares (COUTINHO-SILVA & OJCIUS, 2012).

Em 2010, Correa *et al*. demonstrou que a ativação do receptor P2X7 em macrófagos infectados com *T. gondii* induz a diminuição da carga parasitária pela fusão lisossômica com o vacúolo parasitóforo e produção de ROS. Em estudos utilizando camundongos nocautes para o receptor P2X7, foi observado que essa condição aumenta a suscetibilidade à infecção aguda pelo *T. gondii*. Esse fenômeno está diretamente associado a uma deficiência na resposta imunológica, pois não há aumento dos níveis de citocinas pró-inflamatória como IL-12, INF-γ, IL-1-β e TNF-α após a infecção. Além disso, foi visto que na ausência do receptor P2X7, a infecção por *T. gondii* se dissemina de maneira descontrolada, levando a um grave comprometimento de órgãos como o fígado e baço (CORREA *et al*., 2017).

Em 2017, Moreira-Souza *et al*. demonstrou que a ativação do receptor P2X7 por ATP extracelular inibe o crescimento de *T. gondii* em macrófagos através da ativação do inflamassoma NLRP3 e produção de ROS. Em modelos *in vivo*, animais nocautes para o receptor P2X7 apresentam suscetibilidade aumentada à infecção por *T. gondii* devido a produção prejudicada de citocinas pró-inflamatórias importantes para o controle da infecção. Por isso, ocorre aumento no dano tecidual e altos níveis de carga parasitária nesses animais, sendo possível concluir que a ativação do receptor P2X7 é crucial para auxiliar na resposta imune eficiente contra a infecção por esse parasita (SAVIO *et al*., 2018).

O conhecimento acerca da infecção do intestino delgado por *T. gondii* e o papel do receptor P2X7 nesse contexto é escasso. Por isso, o presente trabalho se propõe a estudar o

papel do receptor P2X7 em modelo murino de ileíte induzida pela ingestão oral de cistos de *T. gondii*.

OBJETIVOS

Objetivo Geral

Investigar o papel do receptor P2X7 na ileíte induzida pela infecção oral com cistos de *Toxoplasma gondii* em modelo murino.

Objetivos específicos

1. Avaliar a expressão do receptor P2X7 no íleo após a infecção com cistos de *T. gondii*;

2. Identificar a extensão da lesão e a carga parasitária no íleo de animais WT e nocautes para o receptor P2X7 ($P2X7^{-/-}$) após a infecção com cistos de *T. gondii*;

3. Quantificar o nível de moléculas e mediadores inflamatórias no soro e no íleo de animais WT e $P2X7^{-/-}$ após a infecção com cistos de *T. gondii*;

4. Analisar a motilidade intestinal dos animais WT e $P2X7^{-/-}$ após a infecção com cistos de *T. gondii*;

5. Verificar a expressão de genes inflamatórios no íleo dos animais WT e $P2X7^{-/-}$ após a infecção com cistos de *T. gondii*;

6. Estudar a microbiota fecal dos animais WT e $P2X7^{-/-}$ após a infecção com cistos de *T. gondii*.

ARTIGO PRINCIPAL NA ÍNTEGRA

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Article

P2X7 Receptor Modulation of the Gut Microbiota and the Inflammasome Determines the Severity of *Toxoplasma gondii*-Induced Ileitis

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Abstract: In mice, oral *Toxoplasma gondii* infection induces severe ileitis. The aim of the present study was to investigate the impact of the P2X7 receptor (P2X7) on the inflammatory response to *T. gondii*-induced ileitis. Cysts of the ME49 strain of *T. gondii* were used to induce ileitis. The infected mice were euthanized on day 8 and ileal tissue and peripheral blood were collected for histopathological and immunohistochemical analyses. Ileal contractility, inflammatory mediators, inflammasome activation, quantitative PCR analysis of gene expression, and fecal microbiota were assessed using appropriate techniques, respectively. The infected P2X7−/− mice had greater disease severity, parasitic burden, liver damage, and intestinal contractility than the infected wild-type (WT) mice. Infection increased serum IL-6 and IFN-γ and tissue caspase-1 but not NLRP3 in P2X7−/− mice compared to WT mice. Bacteroidaceae, Rikenellaceae, and Rhodospirillales increased while Muribaculaceae and Lactobacillaceae decreased in the infected WT and P2X7−/− mice. Bacteroidia and Tannerellaceae increased in the P2X7−/− mice with ileitis. By contrast, Clostridiales and Mollicutes were absent in the P2X7−/− mice but increased in the WT mice. P2X7 protects mice against *T. gondii* infection by activating the inflammasome and regulating the local and systemic immune responses. Specific gut bacterial populations modulated by P2X7 determine disease severity.

Keywords: gut microbiota; inflammasome; purinergic signaling; P2X7 receptor; *Toxoplasma gondii* ileitis

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1. Introduction

Toxoplasma gondii is an intracellular parasite in Phylum Apicomplexa. It causes one of the most common human parasitic infections and has been extensively utilized in murine infection models [1]. Although chronic *T. gondii* infection is estimated to involve almost

one-third of the human population, most infections are asymptomatic in healthy hosts but can become severe in immunocompromised individuals [2]. After oral ingestion, the walls of the *T. gondii* cyst disrupt in the host stomach, tachyzoites are released, and they disseminate through the distal ileum, where they induce inflammation [3]. In mice, oral *T. gondii* infection causes ileitis, which resembles human inflammatory bowel disease (IBD) and has been proposed as an experimental model of Crohn's disease ileitis [4]. In mice orally exposed to *T. gondii*, inflammatory cells rapidly accumulate and create a milieu predominated by the Th1-type immune response [5]. The immune and inflammatory responses against *T. gondii* infection are vital for the control of parasite replication and proliferation within host tissues. Nevertheless, the production of proinflammatory mediators can also lead to tissue damage and is pivotal in disease pathogenesis.

Tissue injury always releases adenosine triphosphate (ATP), which activates purinergic receptors. The P2X7 receptor (P2X7) has been extensively studied and occurs in epithelial and immune cells [6]. The ATP-P2X7 pathway modulates cytokine production, inflammatory mediator secretion, inflammasome activation, and other intracellular signaling pathways. Hence, it participates in the pathogenesis of several inflammatory diseases [7]. P2X7 expression in immune cells has been implicated in the control of *T. gondii* and other intracellular infections in humans. P2X7 mutations are associated with augmented susceptibility to *T. gondii* [8]. In vivo, *T. gondii* infection rapidly upregulates epithelial C-C motif chemokine ligand 5 (CCL5) and recruits dendritic cells to the epithelium in WT but not P2X7−/− mice [9]. In another in vivo study, *T. gondii* infection in human fetal small intestinal epithelial cells revealed the roles of the P2X7/NLRP3 pathway in promoting IL-1β secretion and inhibiting *T. gondii* proliferation [10]. A study on peritoneal and bone marrow-derived macrophages showed that the control of *T. gondii* via P2X7 depends on the canonical NLRP3 inflammasome. The latter increases IL-1β production via caspase-1 activity, induces mitochondrial reactive oxygen species generation, and eliminates parasites [11]. The role of the P2X7 in *T. gondii* infection-induced intestinal disease has been previously investigated. Nevertheless, several questions about this process remain unanswered.

The precise molecular mechanisms involved in toxoplasmosis-induced ileitis are not fully understood. Nonetheless, there is evidence to suggest that the gut microbiota triggers the inflammatory process through lipopolysaccharide (LPS)-induced TLR-4 signaling [12]. Another experimental study identified changes in the gut microbiota that were associated with the development of *T. gondii* ileitis [13]. In addition, the indigenous gut microbiota was shown to protect the host against certain infections [14,15]. Thus, we hypothesized that compositional changes in the gut microbiome may precede the onset of *T. gondii*-induced ileitis. In the present study, we used an in vivo experimental mouse *P2X7R* knockout model to clarify the potential roles of the P2X7 receptor in regulating the events promoting *T. gondii*-associated ileitis and influencing the gut microbiota.

2. Materials and Methods

2.1. Animal Study Ethics Statement

The Institutional Animal Care Committee of the Health Sciences Centre of the Federal University of Rio de Janeiro approved the care and use of the animals and procedures reported in the present study (approval ID: 086/18) in accordance with the guidelines of Animal Research: Reporting of In Vivo Experiments (ARRIVE) (www.nc3rs.org.uk/ARRIVE (accessed on 10 January 2022)) developed by the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs).

2.2. Animals

All mice in each experimental group of this study were housed in the same rack-mounted wire cage. It had sufficient space to accommodate all mice and reduce the heterogeneity in gut microbiota composition between individuals [16]. Standard laboratory pelleted formula and autoclaved water were provided ad libitum. Female C57BL/6 wild type (WT) and P2X7 knockout (P2X7−/−) mice aged 6–8 weeks were procured from The

Jackson Laboratory (Bar Harbor, ME, USA) and maintained at 20–24 °C under a 12 h/12 h light-dark cycle and specific pathogen-free conditions. Carworth Farm mice (CF-1) were obtained from the State University of Campinas (São Paulo, Brazil) and used to maintain *Toxoplasma gondii* passage. The CF-1 mice were orally infected by gavage with a maximum volume of 100 mL phosphate-buffered saline (PBS) containing 50 brain cysts of nonvirulent type II *T. gondii* ME-49 strain. The WT and P2X7−/− mice were orally infected by gavage with ten ME-49 cysts. Orally infected mice were monitored once weekly for one month after infection. Susceptibility to infection was determined based on mouse survival.

2.3. Maintenance of T. gondii Cysts

T. gondii was maintained using Swiss mice aged 6–8 weeks. Each mouse was orally infected by gavage with 10 ME-49 cysts. After two months, the mice were euthanized in a CO_2 chamber. Death was confirmed by cervical dislocation. The brains were excised and macerated in PBS. The cysts in the brain homogenates were counted and used either to infect other mice and maintain the parasite reservoir or to induce ileitis.

2.4. Experimental Design

After an acclimation period of one week, the WT and P2X7−/− mice were randomly allocated to the experimental groups and orally infected by gavage with 10 ME-49 cysts. Body weight was measured daily and used as a clinical parameter of ileitis development. On day 8 of infection, the mice were euthanized in a CO_2 chamber. Blood samples were collected by cardiac puncture and the small intestines were excised, measured, weighed, and washed with saline. Ileal fragments were collected and processed for the subsequent analyses. The mice were enumerated with G*Power (https://g-power.apponic.com (accessed on 8 February 2021)) and two-way ANOVA. The input settings were Cohen's F = 0.3, type I error = 0.05, power = 0.8, and numerator df = 80.

2.5. Ileal Parasite Load

Details of parasite load determination are described in the Supplementary Materials.

2.6. Aminotransferase Assays

The blood was centrifuged at 9000× *g* for 5 min. Then 20 µL serum was added to each well of a 96-well plate. Each well contained 100 µL Master Reaction Mix (Bioclin, Belo Horizonte, Brazil) consisting of enzyme mix, developer, substrate, and assay buffer. The plates were shielded from light, mixed, and incubated at 37 °C for 5 min. Absorbance was measured at 340 nm in a SpectraMax plate reader to detect aspartate transaminase (AST) and alanine transaminase (ALT) according to the manufacturer's protocol.

2.7. Cytokine Measurements

The blood was centrifuged at 9000× *g* for 5 min. The plasma was collected and its cytokine content was measured with a Cytometric Bead Array Mouse Th1/Th2/Th17 Cytokine Kit (BD Biosciences, San Jose, CA, USA) in a FACSCalibur flow cytometer (BD Biosciences). The results were generated and analyzed with BD CBA Analysis software (BD Biosciences). The total protein content of the biopsy specimens was estimated with a Pierce bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA) and used to normalize the results.

2.8. Histological Analysis

Ileal samples were immediately fixed in 40 g/L formaldehyde-saline for ≥24 h and embedded in paraffin. The ileal tissues were cut into 5-µm sections, stained with hematoxylin and eosin (H&E), and microscopically examined. Histological parameters including ulceration, hyperplasia, and inflammatory infiltration were evaluated to calculate the inflammatory score. Details of the histological evaluation are described in the Supplementary Materials.

2.9. Immunohistochemistry (IHC) and Apoptosis Assessment

Details of the IHC experiments are described in the Supplementary Materials.

2.10. Nitric Oxide (NO) Production and Myeloperoxidase Activity Assessment

Details of the NO and myeloperoxidase activity analyses are described in the Supplementary Materials.

2.11. Ileal IL-1β Measurement

Ileal samples were collected, macerated in PBS on ice, and centrifuged at 400× *g* for 5 min. The supernatants were collected and used to measure the ileal IL-1β concentrations. The total protein content of the ileal fragments was quantified with a Pierce BCA protein assay kit (Thermo Fisher Scientific) and used to normalize the results. The ileal IL-1β levels were quantitated using a commercially available sensitive enzyme-linked immunosorbent assay (ELISA) method.

2.12. RNA Isolation, cDNA Synthesis, and qRT-PCR

RNA was extracted from the ileal fragments with TRIzol reagent (Thermo Fisher Scientific, Wilmington, DE, USA). The cDNA was constructed with a high-capacity kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. RT-qPCR was performed on 1 µg cDNA. Gene expression levels were validated by qRT-PCR. RT-PCR was performed in an ABI Prism 7500 (Applied Biosystems, Foster City, CA, USA) with a CustomTaqMan® Array Gene Signature Plate (Thermo Fisher Scientific). Further details on the qRT-PRC and the primers are described in the Supplementary Materials.

2.13. Contractile Activity of Longitudinal Smooth Muscle in the Ileum

Details of the contractile activity analysis are described in the Supplementary Materials.

2.14. Fecal Microbiota Analysis

Details of the fecal microbiota analysis by high-throughput 16S rRNA sequencing are described in the Supplementary Materials.

2.15. Statistical Analysis

Statistical analyses were performed in Graph Pad Prism v. 9.1.2 (GraphPad Software, San Diego, CA, USA). A two-tailed *t*-test was used for pairwise comparisons. Multiple comparisons were performed by one-way ANOVA followed by the post hoc Tukey's test. The results are means ± standard deviations (SD) or standard errors of the mean (SEM). Survival data are presented in the form of Kaplan–Meier survival curves and were analyzed by the log-rank test. All tests were two-tailed and differences between treatments were considered statistically significant at $p < 0.05$.

3. Results

3.1. The P2X7 Receptor Is Overexpressed in the Ileum Following T. gondii Infection

To investigate P2X7 expression during intestinal inflammation induced by *T. gondii*, C57BL/6 WT and P2X7−/− mice were orally infected with ten ME-49 cysts. The expression of the P2X7 receptor was analyzed by IHC in the intestinal sections on day 8 post-infection. The results showed that the P2X7 receptor was significantly upregulated in the ileal mucosae of *T. gondii*-infected mice compared to the uninfected controls (Figure 1A,B). We analyzed molecular-level P2X7 receptor expression to confirm this finding. The P2X7 mRNA level was significantly higher in the ileal samples from *T. gondii*-infected mice than it was in those from the uninfected controls (Figure 1C).

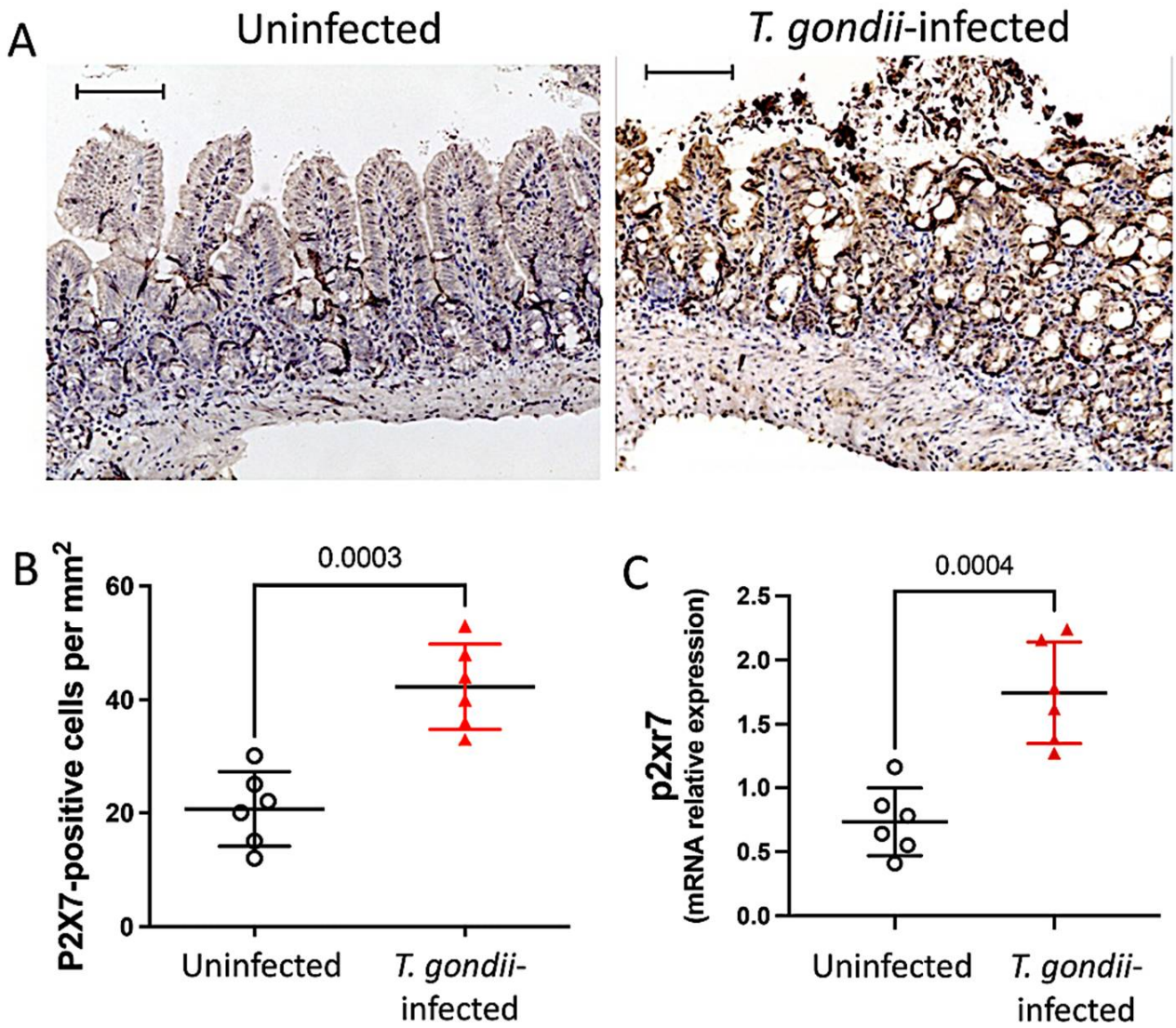

Figure 1. *T. gondii* infection upregulates P2X7 in mouse ileum. Immunohistochemical staining with anti-P2X7 antibody was performed for computerized quantification and analysis of protein distribution in the ileum in the presence and absence of *T. gondii* infection. Bars = 50 µm. Numbers of positive cells/mm^2 are presented as means ± standard deviation (SD) (**A**,**B**). mRNA levels in ileal samples were measured by quantitative real-time PCR and confirmed that *T. gondii* infection upregulates P2X7 in the ileum. Data are means ± SD (**C**). Analysis by unpaired *t*-test. Significant differences are presented.

3.2. The P2X7 Receptor Protects the Host against T. gondii Infection

3.2.1. Survival Curve

A survival curve was plotted to characterize the roles of the P2X7 receptor in *T. gondii*-induced ileitis. Susceptibility was evaluated for 30 d after oral infection with 10 ME-49 cysts. The P2X7−/− mice were relatively more susceptible to oral infection than the WT mice (Figure 2A).

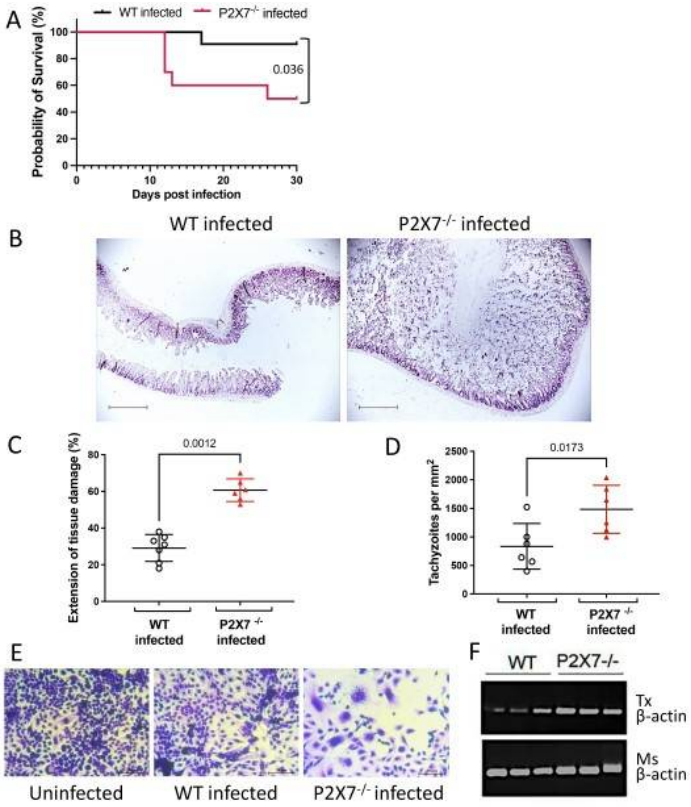

Figure 2. The P2X7 receptor promotes mouse survival by limiting tissue damage and parasite load. The survival curves showed that the infected P2X7−/− mice had significantly higher mortality than the infected WT mice from day 10 onwards (**A**). Data are means ± SD of 11 (WT) and 10 (P2X7−/−) mice/group. Histopathological analysis by hematoxylin and eosin (H&E) staining of the ileum shows more extensive tissue damage in the infected P2X7−/− mice than the infected wild-type (WT) mice. Data are means ± SD of seven (WT) and six (P2X7−/−) mice/group. (**B**,**C**). The number of tachyzoites was higher in the ileal tissue of the infected P2X7−/− mice than the infected WT mice (**D**). Data are means ± SD of six mice/group. ANOVA was performed and followed by the post hoc Tukey's test. A plaque image of the monolayer showed that the cells treated with the ileal supernatant of infected P2X7−/− mice were more severely damaged than those subjected to the ileal supernatant of infected WT mice. Representative micrograph of three mice/group (**E**). The mRNA levels in the ilea of the infected mice were measured by RT-PCR. *T. gondii* β-actin was more abundant in the infected P2X7−/− mice than the infected WT mice. Amplification products of three mice/group (**F**).

3.2.2. Tissue Damage and Parasite Loading

Histological analyses with H&E staining were performed on the ileum to assess the severity of ileitis induced by *T. gondii* infection (Figure 2B). The *T. gondii* infection damaged the ileal mucosa to a significantly greater extent in the P2X7−/− than in the WT mice. The injuries were concentrated in the terminal ilea (Figure 2C).

We investigated the presence of the parasite in the intestines of the infected animals to identify the reasons for exacerbated inflammation. H&E staining showed that there were more tachyzoites in the ilea of the P2X7−/− mice than there were in those of the WT mice (Figure 2D). We examined the parasite load by cell monolayer disruption. An intact LLCM-K2 cell monolayer was cultured with 200 µL WT or P2X7−/− mouse ileum lysate for one week. Rapid monolayer disruption was observed in the presence of the parasites recovered from the P2X7−/− ileum lysate (Figure 2E). We validated the parasite load assay by quantifying parasite β-actin in the ileal fragments via semiquantitative RT-PCR. There was more *T. gondii* β-actin in the ileal fragments of the P2X7−/− mice than in those of the WT mice (Figure 2F).

No other significant differences between the WT and P2X7−/− mice in terms of intestinal injury during *T. gondii* infection were noted. Severe ileal inflammation was characterized by epithelial intestinal extrusion and dense inflammatory cell infiltration, necrosis, and ulceration. The inflammatory scores for the terminal ilea did not significantly differ between the infected WT and P2X7−/− mice (Supplementary Figure S1A,C). In both types of infected mice, the numbers of Paneth cells and their granular contents were reduced (Supplementary Figure S1B,D,E). The proportions of differentiated goblet cells were significantly reduced in both types of infected mice (Supplementary Figure S2A,C). The increases in collagen fiber density that occurred in response to *T. gondii* infection did not significantly differ between the infected WT and P2X7−/− mice (Supplementary Figure S2B,D). We measured ki67-cell proliferation marker expression and apoptosis by TUNEL assay to evaluate cell turnover in the ileal mucosae. Ki67 expression did not significantly differ among treatment groups. Nevertheless, the rate of apoptosis was significantly higher in the *T. gondii*-infected mice than the untreated control mice but did not significantly differ between the infected WT and P2X7−/− mice (Supplementary Figure S3A–D).

3.3. P2X7 Modulates Morbidity and the Inflammatory Response in T. gondii Infection

3.3.1. Body Weight and Liver Aminotransferases

Mouse body weight was measured for 8 d post-infection to determine the clinical consequences of *T. gondii*-induced ileitis. The uninfected mice presented with no significant variation in body weight. During *T. gondii* infection, however, the infected WT mice and the infected P2X7−/− mice showed significant body weight loss (Figure 3A). The plasma aspartate transferase (AST) and alanine transferase (ALT) levels were measured to assess liver damage and dysfunction. The infected P2X7−/− mice presented with higher plasma ALT and AST levels than the infected WT mice (Figure 3B,C).

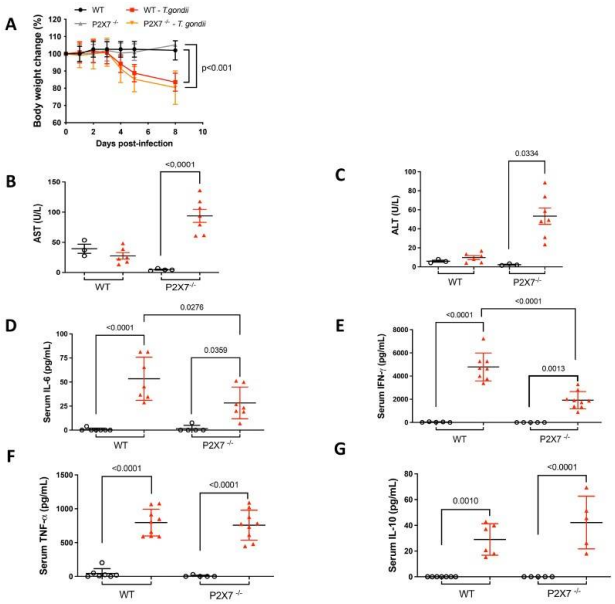

Figure 3. The P2X7 receptor protects against liver damage and inflammatory cytokine production in *T. gondii*-infected mice. Compared to uninfected mice, those with *T. gondii* infection underwent progressive weight loss. Body weight was monitored in 5–10 mice/group and the *p*-value was determined by a log-rank test (**A**). Serum aminotransferases were measured for 3–7 mice/group. There were increased AST (**B**) and ALT (**C**) levels in the P2X7−/− infected mice. Serum IL-6 (**D**), IFN-γ (**E**), TNF-α (**F**), and IL-10 (**G**) levels were measured for 7–9 mice/group and all were elevated in the *T. gondii*-infected animals. However, IL-6 (**D**) and IFN-γ (**E**) increased to a significantly less extent in the infected P2X7−/− mice than in the infected WT mice. Data were subjected to ANOVA and the post hoc Tukey's test was used for multiple comparisons. Data are means ± standard error of the mean (SEM) and significant differences are shown.

3.3.2. Systemic Inflammatory Response

Systemic proinflammatory cytokines limit *T. gondii* infection, replication, and dissemination. We used flow cytometry to measure the proinflammatory cytokine levels in the peripheral blood. IL-6, IFN-γ, TNF-α, and IL-10 were all upregulated in both groups of infected mice. However, the IL-6 and IFN-γ levels were lower in the sera of infected P2X7−/− mice than in those of the infected WT mice (Figure 3D–G).

3.3.3. Mucosal Inflammatory Response and Caspase-1 Activation

Analysis of the inflammatory cell infiltrates within the ileal samples showed high CD4-positive and CD11b-positive cell densities in the lamina propria of the infected WT and P2X7−/− mice (Supplementary Figure S4A,B,D,E). Elevated caspase-1-positive cell densities were also observed in the lamina propria of the infected WT and P2X7−/− mice (Supplementary Figure S4C,F). The ileal extracts of the mice infected with *T. gondii* presented with increased myeloperoxidase activity (Supplementary Figure S5).

3.4. Increased Ileal Contractility Is Enhanced in the Absence of the P2X7 Receptor in T. gondii Infection

As the P2X7−/− mice presented with substantial ileal injury, we investigated whether *T. gondii* infection-induced inflammation affects intestinal physiology. We found no difference between treatment groups in terms of the lengths of their small intestines (data not shown). However, the small intestine weight was higher in the infected P2X7−/− mice than the infected WT mice (Figure 4A). Acetylcholine caused a dose-dependent increase in the contraction of ileal tissue excised from infected WT and P2X7−/− mice as well as uninfected control mice (Figure 4B). However, there was a significant difference in E_{max} for ACh-induced contraction between the WT mice (1.79 ± 0.12 mN, $n = 4$) and the P2X7−/− mice (3.71 ± 0.38 mN, $n = 3$, $p = 0.01$). Hence, ACh induced stronger ileal contraction in the uninfected P2X7−/− mice than in the uninfected WT mice (Figure 4C). Moreover, the infected WT mice presented with stronger ileal contractions (2.91 ± 0.22 mN, $n = 5$) than the uninfected WT mice (1.79 ± 0.12 mN, $n = 4$). The ileum of infected P2X7−/− mice showed the highest contraction in response to ACh (4.39 ± 0.46 mN, $n = 3$, $p = 0.009$) (Figure 4C).

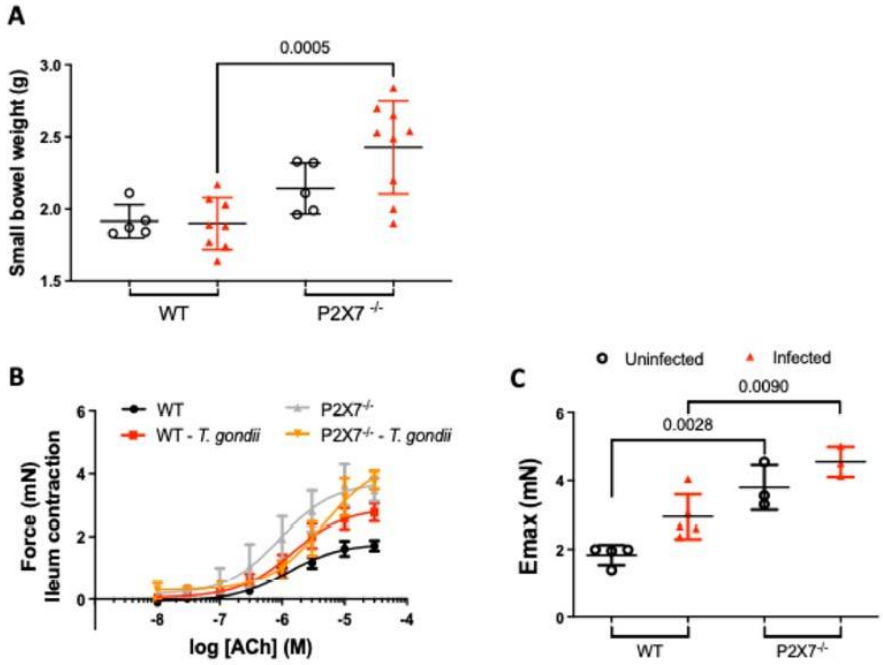

Figure 4. Role of the P2X7 receptor in gut physiology. P2X7 receptor deficiency results in increased intestinal weight in infected mice. Data are means ± standard deviation (SD) of 5–8 mice/group. They

were subjected to ANOVA and the post hoc Tukey's test was used for multiple comparisons. (**A**). Concentration–response curves for contraction induced by ACh (0.01–30 μM) in an ileal segment. Contraction was ACh dose-dependent in the ilea excised from infected wild-type (WT) and P2X7−/− mice as well as uninfected mice. Data are means ± standard error of the mean (SEM) of 3–5 mice/group. They were subjected to ANOVA and the post hoc Tukey's test was used for multiple comparisons. (**B**). Maximal response (E_{max}) to Ach-induced contraction showed that infected P2X7−/− mice had relatively increased ileal contraction. Data are means ± SD of 3–5 mice/group (**C**). Significant differences are shown.

3.5. Inflammasome-Mediated Immune Response against T. gondii Infection

3.5.1. RT-qPCR

The P2X7 receptor activates the inflammasome, which, in turn, secretes IL-1β. This cytokine was shown to control macrophage infection and cerebral toxoplasmosis in different models [17]. We measured the mRNA levels of the inflammasome components and the protein levels of IL-1β in the ilea of *T. gondii*-infected WT and P2X7−/− mice. The NLRP3, NLRP6, Caspase-1, and IL-1β mRNA levels were higher in the infected WT mice than in the infected P2X7−/− mice. By contrast, the AIM2 inflammasome mRNA level was significantly lower in the infected P2X7−/− mice than in the infected WT mice (Figure 5A–F).

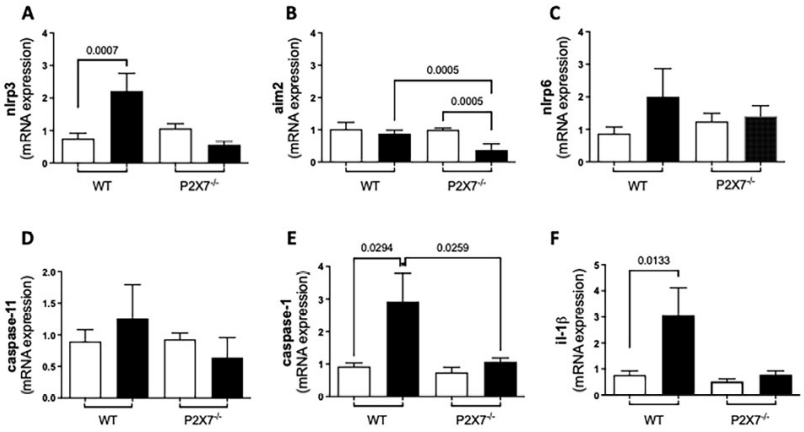

Figure 5. *T. gondii* infection promotes an imbalance in the mRNA expression of inflammasome components measured by quantitative real-time PCR in ileal samples. Significant increases in *NLRP3* (**A**), *Caspase-1* (**E**), and *IL-1β* (**F**) were observed in infected wild-type (WT) mice but not in infected P2X7−/− mice. In contrast, *AIM2* decreased following infection to lower levels in P2X7−/− mice than WT mice (**B**). *NLRP6* (**C**) and *Caspase-11* (**D**) did not significantly change. Data are means ± SEM of 8–13 mice/group. They were analyzed by ANOVA and the post hoc Tukey's test was used for multiple comparisons. Significant differences are presented.

3.5.2. The P2X7 Receptor Modulates NLRP3 and IL-1β Upregulation and NO Downregulation after *T. gondii* Infection

As *T. gondii*-induced ileitis upregulated inflammasome mRNA, we measured the protein expression levels of NLRP3 and IL-1β in the ileal tissue. After *T. gondii* infection, NLRP3 expression was higher in the ileal mucosa of infected WT mice than in infected P2X7−/− mice (Figure 6A,B). The IL-1β concentrations were significantly lower in the ileal lysates of infected P2X7−/− mice than they were in those of infected WT mice (Figure 6C). On the other hand, the production of inflammatory mediators such as nitric oxide (NO) was greater in infected P2X7−/− mice than in uninfected P2X7−/− mice (Figure 6D). Therefore, *T. gondii* infection caused an unbalanced ileal inflammation response in the absence of the P2X7 receptor.

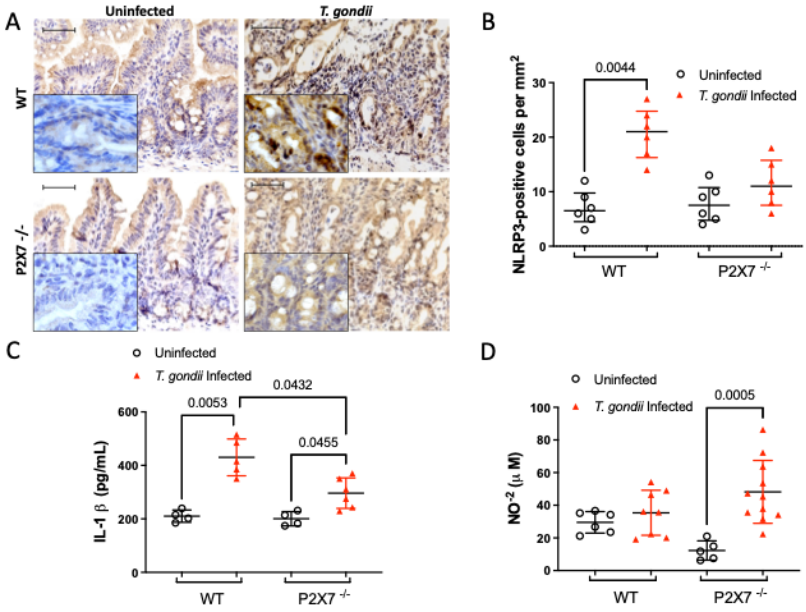

Figure 6. The P2X7 receptor modulates NLRP3 expression and IL-1β and NO production in the ilea of *T. gondii*-infected mice. Immunohistochemical staining showed significant NLRP3 upregulation in infected wild-type (WT) mice but not in infected P2X7−/− mice. Inserts represent magnified micrographs obtained under oil immersion. Data are shown as the means ± standard deviation (SD) of 6–7 mice/group (**A**,**B**). IL-1β was significantly more upregulated in ileal lysates from infected WT mice than it was in those from infected P2X7−/− mice. Data are means ± SD of 4–6 mice/group (**C**). NO production was measured by the Greiss method. NO production was significantly higher in the infected P2X7−/− mice than in the infected WT mice. Data are means ± SD of 5–11 mice/group (**D**). They were subjected to ANOVA and the post hoc Tukey's test was used for multiple comparisons.

3.6. The P2X7 Receptor Modulates the Microbiota Associated with T. gondii-Induced Ileitis

Twenty-four ileal samples were subjected to Deblur quality control clean-up. The values (means ± SD) were as follows: control WT: 52,780 ± 1889; induced ileitis WT: 75,335 ± 2834; control P2X7−/−: 43,565 ± 935; and induced ileitis P2X7R−/−: 111,717 ± 1682. High-throughput sequencing generated 1011229 16S rRNA sequences ~85 nt in length. The reads were then segregated into 215 operational taxonomic units (OTUs). Estimators of α-diversity were calculated based on the Shannon index. Although no significant difference in α-diversity was detected among the groups, we observed a trend toward a decreased diversity in the ileitis-induced groups (Figure 7A). A principal coordinate analysis (PCoA) revealed clustering of the P2X7−/− samples (blue and green) away from the WT samples (red and orange). Ileitis induction caused unique structural changes in the fecal microbiota. Most induced ileitis samples shifted away from the control samples highlighted with colored oval forms. However, the WT controls were unevenly distributed. Hence, there was heterogeneity within that group and a more comprehensive and accurate analysis could not be performed. The fecal microbiota differed between the control and ileitis-induced mice and between the WT and P2X7−/− control mice (Figure 7B). A Venn diagram showed that most OTUs (67%) were conserved and were shared among groups. Similar superimpositions were observed for the WT mice (76%) and P2X7−/− mice (81%) and for the ileitis-induced mice (78%) and the control mice (73%) (Figure 7C). In all treatment groups, the fecal microbiota comprised mainly the phyla Bacteroidetes and Firmicutes followed by the less abundant phyla Proteobacteria and Epsilonbacteraeota, among others. No significant differences were detected among treatment groups in terms of their bacterial phyla (Figure 7D). Identification of the hierarchies within the taxonomic system enabled us to detect several distinct compositional patterns in the treatment groups. The relative microbial abundances significantly differed between the ileitis-induced and control mice and between the WT and P2X7−/− mice (Supplementary Figures S6–S9). In the WT mice,

T. gondii-induced ileitis induced several changes in the gut microbiota, including an increased relative abundance in Rhodospirillales order (Proteobacteria phylum), Bacteroidaceae family (Bacteroidetes phylum), Gastranaerophilales order (Cyanobacteria phylum), and a reduction in Burkholderiaceae family (Proteobacteria phylum), Eggerthellaceae family (Actinobacteria phylum), and Desulfovibrionaceae family (Thermodesulfobacteriota phylum), compared with WT uninfected mice ((Supplementary Figure S6). In the P2X7−/− mice, *T. gondii*-induced ileitis induced several clear changes in the gut microbiota, including an increased relative abundance in Bacteroidaceae family and Bacteroidales order (Bacteroidetes phylum), Lachnospiraceae family (Firmicutes phylum), and Gastranaerophilales order (phylum Cyanobacteria), Saccharimonadaceae family (Saccharibacteria phylum), Rikenellaceae order (Bacteroidetes phylum), Clostridiales vadin class (Firmicutes phylum), Rhodospirillales order (Proteobacteria phylum), and reductions in Burkholderiaceae family (Proteobacteria phylum), Prevotellaceae family (Bacteroidetes phylum), and Muribaculaceae family (Bacteroidetes phylum) compared with P2X7−/− uninfected mice (Supplementary Figures S7 and S8). Comparing the uninfected mice, several clear changes in the indigenous gut microbiota were detected in P2X7−/− mice, including a reduction in the relative abundance in Saccharimonadaceae family (Saccharibacteria phylum), Clostridiales vadin class and Lactobacillaceae family (Firmicutes phylum), and increases in Bacteroidales order, Prevotellaceae family, and Tannerellaceae family (Bacteroidetes phylum), compared with WT mice (Supplementary Figure S9). The Firmicutes:Bacteroidetes ratios did not significantly differ among treatment groups (Supplementary Figure S10).

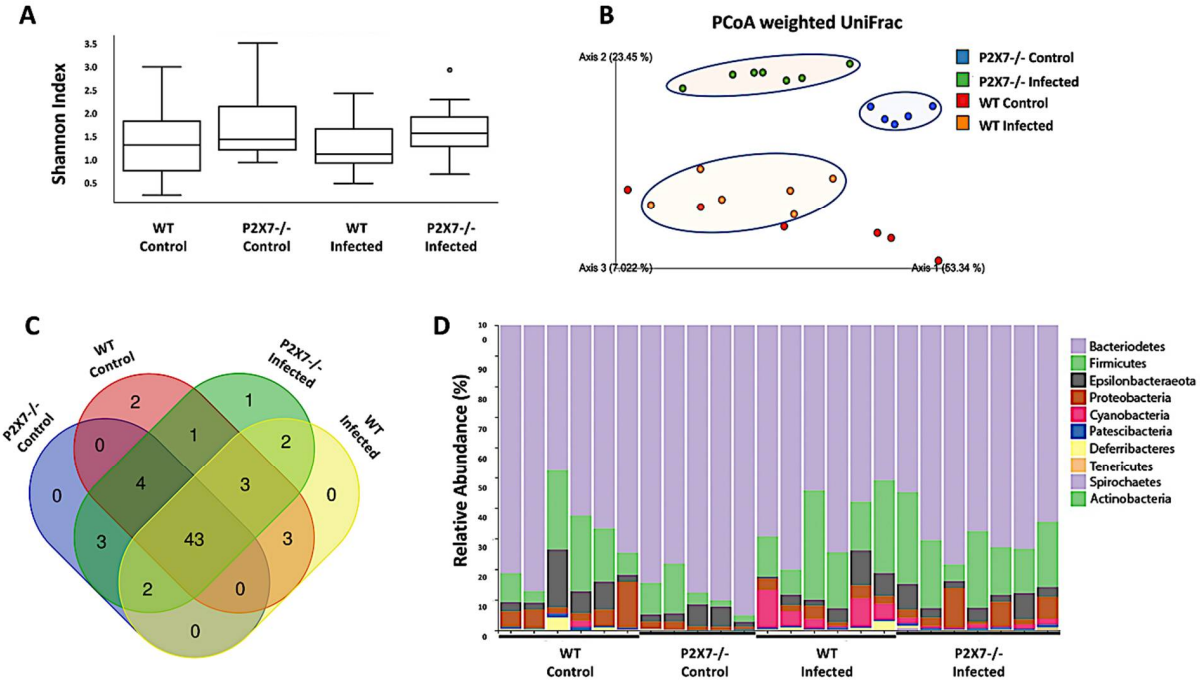

Figure 7. *T. gondii* infection and the P2X7 receptor modulate the intestinal microbiota. Alpha-diversity analysis using the Shannon index showed an upward trend in fecal microbial diversity in the absence of the P2X7 receptor (**A**). β-diversity analysis using weighted UniFrac PCoA clusters P2X7−/− away from WT mice and *T. gondii*-infected mice away from control mice (**B**). Venn diagram showing logical associations among groups (**C**). Differential abundance analysis of taxonomic profiles depicting phylum-level microbial composition (**D**). Sequencing was performed on samples from two independent experiments involving 2–5 mice/group.

4. Discussion

In the present study, we investigated whether purinergic signaling via P2X7 is involved in the pathogenesis of a murine model of *T. gondii*-induced ileitis. P2X7 deficiency markedly increased the morbidity and mortality associated with oral *T. gondii* infection.

We demonstrated that P2X7-deficient mice infected with *T. gondii* had a greater intestinal parasite burden, more extensive intestinal inflammation, and higher intestinal contractility than WT mice infected with *T. gondii*. The changes in the systemic and local inflammatory responses observed in the P2X7-deficient mice infected with *T. gondii* were associated with increased NO production and decreased inflammasome activation. Moreover, substantial changes in gut bacterial abundance and diversity were detected in the P2X7-deficient mice and associated with increased severity of *T. gondii* infection.

The intestinal P2X7 receptor was associated with the severity of inflammation in human IBD [18] and in experimental colitis [19,20] and ileitis [21] models. P2X7 was related to increased susceptibility to *T. gondii*-induced ileitis [22]. A previously established model of chronic *T. gondii* infection was induced by gavage administration of five ME-49 cysts over 30 d [17]. Here, however, we administered 10 ME-49 cysts and the P2X7−/− mice began to die by day 10 after infection, whereas the WT mice began to die by day 18 after infection. Miller et al. (2015) reported exacerbation of *T. gondii*-induced ileitis in P2X7−/− mice but observed no increase in their ileal or splenic parasite loads [22]. However, another study showed that intraperitoneal injection of 20 ME-49 cysts induced ileal dysbiosis and increased nitrate levels in mice [23]. By contrast, the present study showed that infected P2X7−/− mice produced relatively more nitrate than uninfected P2X7−/− mice. Thus, microbial alterations in the intestines of P2X7−/− mice might increase inflammatory mediator production. Nonetheless, we observed relatively more severe tissue damage in the infected P2X7−/− mice, in which the infection resulted in extensive small bowel inflammation, in contrast to WT-infected mice, which showed intestinal inflammation limited to the terminal ileum. One possible explanation is that the mice could not control parasite proliferation. In fact, PCR and plate assays of histopathological intestinal sections confirmed increased numbers of tachyzoites. Uncontrolled parasite loads were reported for a chronic ME-49 infection model. The investigators detected comparatively more cysts and less inflammatory infiltration in the brains of P2X7−/− than WT mice [17].

An acute *T. gondii* infection model was induced by intraperitoneal injection of the RH strain in P2X7−/− mice. The mice presented with increased susceptibility to infection and several pathological alterations such as liver damage, clotting, adherence, and increased parasitic load compared to the WT mice. Regarding the liver, *T. gondii*-infected P2X7−/− mice developed smaller granulomas, but increased parasite load per granuloma [24]. Liver damage in P2X7−/− mice was indicated by elevated aminotransferase levels. The *T. gondii* infection also caused an imbalance in inflammatory cytokine production. IL-6, IFN-γ, IL-1β, and TNF-α are involved in immune cell migration and NK cell, lymphocyte, and monocyte activation. They were upregulated in all *T. gondii*-infected mice but particularly in infected P2X7−/− mice [22]. Proinflammatory cytokines limit *T. gondii* burdens and, therefore, the severity of the infection [25]. However, the serum IL-6 and IFN-γ levels were lower in infected P2X7−/− mice than in infected WT mice in this study. An in vitro study showed that IL-6 levels decreased during pharmacological P2X7 receptor blockade in mouse colonic epithelial (CMT-93) cells subjected to *T. gondii* infection [9]. This finding corroborates our results. In an in vivo mouse model of acute *T. gondii* infection, the P2X7 receptor was responsible for increases in serum IFN-γ greater than those in splenic CD4+ IFN-γ+ cells [24]. During acute toxoplasmosis, the P2X7 receptor participates in the immune response, thereby promoting the production of inflammatory cytokines that limit parasite burdens.

The results of this study showed increased intestinal contractility in mice infected with *T. gondii*. Similarly, a previous study demonstrated that *T. gondii* mediates alterations in the enteric nervous system [26], which regulates intestinal motility [27,28]. However, the present study showed that acetylcholine mediated myogenic intestinal contraction in a dose-dependent manner. This effect was particularly pronounced in *T. gondii*-infected P2X7−/− mice. Discrepancies in the degree of intestinal contractility in the P2X7−/− mice corroborate the results of earlier reports linking P2X7 receptor expression in enteric neurons with the regulation of intestinal motility [29,30]. The mechanisms underlying the changes in

intestinal contractility observed herein are unclear. Nevertheless, we hypothesize that P2X7 deficiency may reduce ATP release from epithelial and enterochromaffin cells which, in turn, lowers inhibitory junctional potentials and, by extension, potentiates the contractions induced by ACh. Under normal conditions, neuronal P2X7 receptors have relatively less control of tonic inhibition on excitatory cholinergic motility [31]. However, in the rat colitis model, the selective P2X7 antagonist A804598 induced a significant enhancement of the contractions of inflamed tissues, which seemed to depend on the NO pathway, since the nitric oxide synthase inhibition blunted the A804598 effect [31]. Here, we found increased NO levels in the ilea of infected P2X7−/− mice. Therefore, our findings are in accordance with the putative role of P2X7 in the regulation of cholinergic-mediated contractions. For this reason, ACh-induced contraction was comparatively stronger in the ilea of the infected P2X7−/− mice than it was in those of the infected WT mice.

Consistent with previous reports on oral *T. gondii* infection, our mouse model also presented with severe intestinal inflammation involving the terminal ileum. In that region, leucocytes accumulated in a predominantly Th1-type immune response, signaling pathways were activated, and several proinflammatory mediators were secreted [32,33]. We detected increases in IL-1β in the infected WT mice but not in the infected P2X7−/− mice. We also observed NLRP3 inflammasome upregulation in the infected WT mice but not in the infected P2X7−/− mice. Although the protein levels of caspase-1 were increased in both WT and P2X7−/− mice following *T. gondii* infection, the respective caspase-1 mRNA increased only in the WT group. This could be explained by the existence of additional pathways for activating caspase-1, such as the direct induction by microorganisms, for example. In a study by Franchi et al., intracellular *Salmonella* or *Listeria* were shown to activate pro-caspase-1 in macrophages, even in the context of P2X7 receptor deficiency [34]. Therefore, we speculate that available cytosolic pro-caspase-1 may be activated, at least in part, by alternative pathways, independent of P2X7. Therefore, the innate immune response against the *T. gondii* insult was upregulated and there might be a mechanistic association between P2X7 and the inflammasome pathways. In contrast to our findings, however, previous in vitro studies showed that *T. gondii* infection may activate the inflammasomes NLRP3, NLRP6, and AIM2 and, by extension, IL-1β secretion in the FHs 74 epithelial cells of the human small intestine and in human leukemia THP-1 monocytic cells whether or not the P2X7 receptor is present [35,36]. Nonetheless, it is difficult to compare and contrast the results generated by in vitro and in vivo studies with different designs and protocols. In the current study, AIM2 inflammasome expression was lower in the P2X7−/− mice than the WT mice and decreased further still after *T. gondii* infection. Taken together, the foregoing results underscore the existence of an intricate network involving P2X7 and the inflammasome pathways, activating caspase-1, and releasing mature IL-1β [37] in response to *T. gondii* infection [10,11].

Here, the inflammatory process was associated with increased oxidative stress and cell death in the intestinal epithelial layer. Similar to previous studies [25,38], we detected elevated NO levels in response to *T. gondii* infection and to a greater extent in P2X7−/− mice than WT mice. The Th1 response is essential in order to promote the production of effector molecules, such as NO that combat protozoan parasites including *T. gondii* [39]. Other investigations showed that *T. gondii* infection per se [40] and Th1-induced nitrate production [23] could induce gut dysbiosis, which may explain the observed dissimilarities in the microbiota among the treatment groups. Alterations in local mucosal immunity align with the structural and physiological damage to the small bowel described for similar experiments [41,42]. These morphophysiological changes resembled those characteristic of Crohn's disease [43].

Loss of the structural cells composing the ileal epithelium adjacent to the mucus-secreting and Paneth cells resulting from *T. gondii* infection might favor both microbial penetration and gut dysbiosis. Disruption of the epithelial barrier might reinforce innate immunity mechanisms against *T. gondii* infection and its associated gut dysbiosis. In the current study, the gut dysbiosis observed after *T. gondii* infection was consistent with

previous investigations reporting reduced microbial diversity and expansion of phylum Proteobacteria, order Rhodospirillales, and facultative anaerobes [23]. Gut dysbiosis observed in the murine *T. gondii* infection model has been attributed to Th1-induced Paneth cell loss and low antimicrobial peptide levels [44,45]. Goblet cell loss may compromise the protective mucous layer and favor bacterial adherence and penetration that occurs mechanically or results from the lack of the trefoil factor family peptides that are co-secreted with mucins [46,47].

The *T. gondii* infection-related compositional changes we detected in both WT and P2X7-deficient mice were not limited to Proteobacteria expansion. In phylum Bacteroidetes, increases in the families Bacteroidaceae and Rikenellaceae were observed in response to *T. gondii* infection. Another study reported the same finding in SAMP1/YitFc mice that had developed spontaneous ileitis [48]. On the other hand, another study using a similar *T. gondii* infection model reported that family Lactobacillaceae (phylum Firmicutes) was reduced in mice presenting with *T. gondii*-induced ileitis [13]. Moreover, *T. gondii*-infected mice exhibited reduced abundance of the family Muribaculaceae (phylum Bacteroidetes). Members of the Muribaculaceae utilize mucus-derived monosaccharides in the gut [49]. Therefore, the results of the current study and previous literature findings support the suggestion that gut dysbiosis following *T. gondii* infection may develop due to several combined mechanisms, including the inflammatory process per se, and the mucosal barrier damage, with reduction in the production of antimicrobial peptides, allowing the selection and penetration of pathogenic microorganisms.

Regarding the potential influence of the P2X7 receptor in the gut microbiota, to our knowledge, a previous study from our group was the first to show a trend toward a higher relative abundance of Cyanobacteria and Spirochaetes in fecal samples from the P2X7−/− mice. Although the findings were associated with a possible protective role against the development of colitis-associated colorectal cancer in the P2X7−/− mice, they lacked statistical significance and the analysis was limited to the phylum level [50]. In this study, the abundance of Family Burkholderiaceae (phylum Proteobacteria) was lower in P2X7-deficient than WT mice and decreased further still after *T. gondii* infection. Similar gut dysbiosis patterns were observed in a chemically induced experimental colitis model [51]. The preceding findings strongly support the occurrence of gut dysbiosis in response to *T. gondii* infection including the expansion of aggressive strains in order Rhodospirillales and families Bacteroidaceae and Rikenellaceae. Hence, the Burkholderiaceae and Muribaculaceae might confer protection against *T. gondii* infection.

Other differences in relative gut microbial abundance may account for the different degrees of severity of *T. gondii* ileitis observed in the P2X7-deficient mice. Orders Bacteroidales and Clostridiales were relatively more abundant in the P2X7-deficient mice and expanded further still after *T. gondii* infection. Similarly, another study evaluating the impact of Toll-like receptor-9 (TLR-9) on the gut microbiota after *T. gondii* infection reported increases in Bacteroidales and Clostridiales in TLR-9-deficient mice. Though TLR-9 is not required to induce *T. gondii* ileitis, it mediates inflammatory changes in the intestinal and extra-intestinal compartments [52]. Another study reported that intestinal Na+/H+ exchanger 3 (NHE3) deletion favors the expansion of proinflammatory bacterial taxa, including Bacteroidaceae, Rikenellaceae, and Tannerellaceae and contraction of the Prevotellaceae [53]. We obtained similar findings for *T. gondii*-infected, P2X7-deficient mice. In fact, a previous study suggested that commensal bacteria can act as molecular adjuvants during *T. gondii* infection. In that study, mucosal innate and adaptive immune responses to dendritic cell stimulation by normal gut microbiota conferred protection against *T. gondii* in the absence of TLR11 [54]. The foregoing findings suggest that compositional changes in the gut microbiota may precede the onset of *T. gondii*-induced ileitis and could explain the relatively greater disease severity in infected P2X7-deficient mice. Taken together, the results from the current study suggest that the gut dysbiosis associated with *T. gondii* infection in our model may represent a secondary phenomenon but may also precede and facilitate the infection.

The present study successfully induced small bowel inflammation and established its association with the P2X7 pathway. Nevertheless, it also had certain important limitations. First, very few experiments and replicates were performed mainly because of technical difficulties associated with mouse care and the aggressiveness of the *T. gondii* infection. Nonetheless, the results of the study revealed several significant differences between treatment groups and high consistency overall. Second, future investigations should consider using P2X7 antagonists and different animal models and experimental protocols. They should collect samples throughout the study to evaluate the dynamic changes that may occur in any of the parameters measured and particularly those involving the gut microbiota. Finally, antibiotics could be administered in certain treatments to help elucidate the mechanistic roles of the gut microbiota in the model and detect any potential synergism with the P2X7 pathway in activating the inflammasome and conferring host protection against *T. gondii* infection.

5. Conclusions

Defective innate immunity in P2X7-deficient mice may render them unable to effectively combat *T. gondii* infection. They mount weaker, less complete inflammatory responses than their WT counterparts. Nonetheless, P2X7-deficient mice infected with *T. gondii* present with wider ileal tissue disruption and more severe consequences than infected WT mice. Therefore, the P2X7 receptor is vital to the host's defense against peroral *T. gondii* infection. It limits parasite-induced intestinal immunopathology, regulates the systemic immune response, and restrains parasite dissemination in the host. Our findings suggest that the P2X7 pathway also shapes the gut microbiota, which may in turn directly or indirectly determine the outcome of the infection challenge. In P2X7 deficiency, indigenous gut microbiota may create an environment favoring increased susceptibility to *T. gondii* infection. Furthermore, abnormal host-microbial interactions in P2X7 deficiency may foster secondary dysbiosis that exacerbates tissue damage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomedicines11020555/s1. Supplementary Materials and Methods; S1.1 Parasitic load in the ileum; S1.2 Histologic analysis; S1.3 Immunohistochemistry; S1.4 Assessment of apoptosis in the ileum; S1.5 Quantitative assessment of ileal sections; S1.6 Myeloperoxidase Activity Assessment; S1.7 Nitric oxide production; S1.8 Analyzing of messenger RNA expression by qRT-PCR; S1.9 Contractile activity of ileal longitudinal smooth muscle; S1.10 Fecal microbiota composition; S2. Supplementary Results; S2.1 Supplementary Figure S1, *T. gondii* infection induces severe ileal inflammation and damage, with a significantly greater extension among the P2X7−/− mice; Supplementary Figure S2. *T. gondii* infection results in intense damage to specialized epithelial cells and deposition of collagen fibers in the ileum; Supplementary Figure S3. *T. gondii* infection is characterized by intense ileitis with marked cell loss; Supplementary Figure S4. *T. gondii*-induced ileitis is characterized by a marked inflammatory cell accumulation and activation in the intestinal mucosa; Supplementary Figure S5. *T. gondii*-induced ileitis is characterized by increased myeloperoxidase activity (MPO) in the affected tissue; Supplementary Figure S6. *T. gondii*-induced ileitis is associated with changes in the gut microbiota of the WT mice; Supplementary Figure S7. *T. gondii*-induced ileitis is associated with changes in the gut microbiota of the P2X7−/− mice; Supplementary Figure S8. *T. gondii*-induced ileitis is associated with changes in the gut microbiota of the P2X7−/− mice; Supplementary Figure S9. The P2X7 receptor modulates the gut microbiota in mice; Supplementary Figure S10. Effect of P2X7 on microbiota composition considering the Firmicutes: Bacteroidetes ratio of fecal samples in *T. gondii*-induced ileitis; S3. References. Refs. [55–61] cited in supplementary material.

Author Contributions: Conceptualization, A.C.A.M.-S., H.S.P.d.S. and R.C.-S.; Data curation, A.C.A.M.-S., H.S.P.d.S., R.C.-S. and T.P.R.; Formal analysis, A.C.A.M.-S., H.F.N., T.P.R., C.M.C., F.T., C.L. and R.C.V.; Funding acquisition, H.S.P.d.S. and R.C.-S.; Investigation, H.F.N., T.P.R. and B.E.R.; Methodology, A.C.A.M.-S., H.F.N., T.P.R., S.R.B.d.S., B.P.D., B.E.R., C.M.C., F.T., C.L., P.T.S., S.L.B.R., K.Q.d.A., C.L.M.S. and R.C.V.; Resources, A.C.A.M.-S., T.P.R., S.R.B.d.S., B.E.R., C.M.C., F.T., P.T.S., S.L.B.R., K.Q.d.A., C.L.M.S. and R.C.V.; Software, A.C.A.M.-S., T.P.R., S.R.B.d.S., B.P.D., C.M.C., F.T., C.L., P.T.S., S.L.B.R., K.Q.d.A. and R.C.V.; Supervision, H.S.P.d.S. and R.C.-S.; Validation, A.C.A.M.-S., H.F.N., T.P.R., S.R.B.d.S., B.P.D., B.E.R., C.M.C., F.T., C.L., P.T.S., S.L.B.R., K.Q.d.A. and C.L.M.S.; Visualization, A.C.A.M.-S., T.P.R., B.P.D., B.E.R., C.L.M.S. and H.S.P.d.S.; Writing—original draft, A.C.A.M.-S., H.F.N. and T.P.R.; Writing—review & editing, H.S.P.d.S. and R.C.-S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The Institutional Animal Care Committee of the Health Sciences Centre of the Federal University of Rio de Janeiro approved the use and care of the animals as well as the procedures reported in this study (approval ID: 086/18).

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Data Availability Statement: Materials such as protocols, analytical methods, and study material are available upon request to interested researchers. The raw data supporting the conclusions of this manuscript will be made available by the authors without undue reservation to any qualified researcher. Submission ID: SUB12533790; BioProject: ID: PRJNA925189.

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Supplementary contents

1. **Supplementary Materials and Methods:**

1.1 Parasitic load in the ileum

Ileal samples were macerated and centrifugated at 250 x g for 5 minutes, and the pellet was resuspended in DMEM media and incubated with LLC-MK2 cells overnight at 37 °C in a humid atmosphere with 5% of the CO_2. After seven days, the cells were fixed and stained with fast panoptic, and the plaque forming was used to quantify the parasitic load. The parasite load was confirmed by semiquantitative PCR. The gDNA from ileum fragments was isolated using the Trizol reagent according to the manufactured instructions. The PCR was performed using the Gotaq green master mix, 2ug of DNA, and *Toxoplasma* β-actin (*Tg* β-actin) or murine β-actin primers. *Tg* β-actin gene- forward primer: GACCTTACCGAGTACATGATGAAG, reverse primer: CCATCGGGCAATTCATAGGAC; Ms β-actin gene- forward primer TATGCCAACACAGTGCTGTCTGG, reverse primer: TACTCCTGCTTGCTGATCCACAT with the following thermocycling program: denaturation 95 ºC for 1 minute, 40 cycles of annealing at 60 ºC for 1 minute, extension at 72 ºC for 5 minutes. The PCR products with *Tg* β-actin 185 bp, and murine β-actin 295 bp, were revealed in a 1.5% agarose gel stained with gel red.

1.2 Histologic analysis

Ileal samples were fixed immediately in 40 g/L formaldehyde saline for at least 24h and embedded in paraffin. The ileum was cut into 5μm sections, stained with hematoxylin and eosin (HE), and analyzed microscopically. To obtain the inflammatory score, histological parameters such as ulceration, hyperplasia, and inflammatory infiltrate were evaluated. The Paneth cells and granules number were measured based on the crypts of the ileum by HE stains. The cells and granules were counted per crypt in at least 10 different areas per tissue section under light microscopy, and the overall number of the cells and granules was expressed by percentage. The periodic acid of Schiff (PAS) technique was used to stain goblet cells within the ileal mucosa. The cells PAS-positive was counted in at least 500 epithelial cells in the crypts and surface epithelium by light microscopy, and the density of goblet cells was defined by percentage. Phosphomolybdic acid picrosirius red dye was used to stain collagen fibers in tissue. At least 5 different areas per tissue section were analyzed under light microscopy connected to a computer-assisted image analyzer.

1.3 Immunohistochemistry

Paraffin sections were cut onto slides pretreated with poly-lysine to characterize Paneth cells and intracellular signaling pathways using the indirect immunoperoxidase technique. Briefly, deparaffinized sections were first incubated at 90°C in 0.01 M sodium citrate buffer (pH 6.0) for 30 min for antigen retrieval. After endogenous peroxidase activity was blocked using 3% hydrogen peroxide in methanol for 30 min, the sections were rinsed in phosphate-buffered saline (PBS) and incubated in 5% bovine serum albumin (BSA) with 0.1% Triton for 30 min. Staining was carried out using the following primary antibodies diluted in 1% BSA: rabbit

For the indirect immunoperoxidase technique, paraffin sections were first cut onto slides pretreated with poly-lysine. After deparaffinization, sections were incubated at 90°C in 0.01 M sodium citrate buffer (pH 6.0) for 30 min for antigen retrieval. Then, the slides were immersed in 3% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity. After being rinsed in phosphate-buffered saline (PBS) containing 0.5 % Tween 20 for 10 min, the tissue sections were incubated with non-immune horse serum for 30 min and, subsequently, with the appropriate antibody. Immunohistochemical staining was performed using the following primary antibodies: rabbit polyclonal anti-P2X7R (1:200) (Alomone Labs, Jerusalem, Israel); rabbit polyclonal anti-ki67 (1:200) (Merck KGaA, Darmstadt, Germany); rabbit monoclonal anti-CD4 antibody (1:50; Santa Cruz Biotechnology Inc., Santa Cruz, CA); rabbit monoclonal anti-CD11b antibody (1:100; ab133357); rabbit polyclonal anti-Caspase-1 antibody 1:500; ab1872); rabbit monoclonal anti-NLRP3 antibody (1:500; EPR23094-1) (all from Abcam, Cambridge,

United Kingdom). Two sections from each sample were incubated with either PBS alone or an isotype monoclonal IgG (concentration matched) and served as the negative controls. After incubation in a humidified chamber overnight at 4°C, the tissue sections were rinsed with PBS and incubated with a Dual Link System-HRP (Dako, Glostrup, Denmark) for 30 min at room temperature. Additional rinsing was followed by development with a solution containing hydrogen peroxide and diaminobenzidine (Dako, Glostrup, Denmark). The preparations were lightly counterstained in Harris's hematoxylin, dehydrated, and mounted in Permount (Fisher Scientific, Pittsburgh, PA, USA).

1.4 Assessment of apoptosis in the ileum

Apoptosis was assessed in ileal sections using a terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay. Samples from all experimental groups were analyzed using an ApopTag Peroxidase In Situ Apoptosis Detection Kit (Millipore Corporation, Billerica, MA, USA). First, paraffin sections were deparaffinized, hydrated, and incubated with a proteinase K solution. After endogenous peroxidase activity was blocked, the slides were covered with equilibration buffer and then incubated with a solution containing TdT enzyme. For the negative controls, we incubated a second section from each sample without the TdT enzyme. For the positive controls, we pretreated samples with DNase I (Sigma-Aldrich, Deisenhofen, Germany). After the reaction was terminated, the specimens were incubated with nonimmune horse serum and then incubated with an anti-digoxigenin antibody peroxidase conjugate. Development and counterstaining of sections were performed exactly as described above for immunohistochemistry. Morphologically preserved TUNEL-positive cells and apoptotic bodies were referred to as apoptotic cells.

1.5 Quantitative assessment of ileal sections

Quantitative analysis of tissue sections (under light microscopy) was carried out using a computer-assisted image analyzer (Leica QWin Plus V 3.5.1, Leica Microsystems, Ltd., Switzerland). Any epithelial and lamina propria cells exhibiting identifiable reactivity distinct from the background were regarded as positive. The results of the quantitative analysis of the cell subsets are expressed as the number of cells per mm^2 of longitudinally sectioned ileum tissues. Two independent observers who were unaware of the experimental data examined all tissue sections and captured images. To increase the resolving power of the microscope, some images were captured under oil immersion.

1.6 Myeloperoxidase Activity Assessment

Ileal samples were collected and frozen at −80◦C until the extraction of myeloperoxidase (MPO) when they were homogenized in potassium phosphate buffer (pH 6.0), frozen and defrosted twice, homogenized again in the potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl-trimethyl-ammonium bromide (Sigma Chemical Co., St. Louis, MO, USA), and centrifuged at 40,000 g for 30min at 4◦C. The supernatants were discarded, and the insoluble pellets were homogenized in the potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl-trimethyl-ammonium bromide. Ten microliters of the supernatants were added to a 96-well plate containing 290 μl of 50mM potassic PBS (pH 6.0), 3 μl of the substrate solution, containing 20 mg/ml o-dianisidine (Sigma Chemical Co., St. Louis, MO, USA), and 3 μl of H2O2 (20mM). The plate components were mixed, and the absorbance was determined at 460 nm for 1min with a spectrophotometer. MPO activity was measured by a standard curve of the samples in units of MPO/mg of colonic samples.

1.7 Nitric oxide production

Fresh ileum samples were collected, macerated in PBS containing 1% of protease inhibitor (Sigma-Aldrich, Darmstadt, St. Louis, MO, USA) on ice, and centrifuged at 1000 x g for 5 minutes. The supernatant was collected and subjected to the colorimetric assay using the Griess method to measure nitric oxide indirectly. Initially, in a 96-well plate, the sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid, both of Sigma-Aldrich, St. Louis, USA) was added to all

experimental samples and wells containing the dilution series for the nitrite standard reference curve. Thereafter, the NED solution (0.1% N-1-naphthyl ethylenediamine dihydrochloride, Sigma-Aldrich, St. Louis, USA, in water) was dispensed into all wells. To ensure the accuracy of NO^{-2} quantification, the reference curve was prepared with the nitrite standard for each assay. The absorbance was measured within 30 min in a plate reader in 550 nm, read using the SpectraMax plate reader.

1.8 Analyzing of messenger RNA expression by qRT-PCR

Ileal fragments were processed to RNA extraction using Trizol reagent (Thermo Scientific, Wilmington, DE, USA) and cDNA construction using the High-capacity kit (Life Technologies, Carlsbad, CA, USA) according to the manufactured instructions. The RT-qPCR was performed using 1ug of DNA. The expression levels of selected genes were validated by quantitative real-time PCR (qRT-PCR). Real-time RT-PCR was performed with an ABI Prism 7500 (Applied Biosystems, Foster City, CA, USA) using a CustomTaqMan® Array Gene Signature Plate (Thermo Scientific, Wilmington, DE, USA), including the p2xr7 forward primer: ATGCCGGCTTGCTGCAGCTGGAACGATGTCTTTCAGTATGAGACA, reverse primer: CCAAGTCTTGTGAAAGGTACAAGAGATGTTCATACCTGGTAAGAT, nlrp3 forward primer: TGTGTGGATCTTTGCTGCG, reverse primer: GTTACTGTGCACATGTAGTGTGTAATAAGG; aim2 forward primer: TTGTCTCCTTCCTCGCACTT, reverse primer: TCGGGTAAGTGTTAACAAGG; nlrp6 forward primer: CCGTGTCCGAGTACAAGAAGAAC, reverse primer: CGCGATGAGCAGCTTGGT; caspase-11 forward primer: TGAAATGCATGTACTGAGAGCAAGG, reverse primer: CAATTGACTTGGGGATTCTG; caspase-1 forward primer: GGAAGCAATTTATCAACTCAGTG, reverse primer: GCCTTGTCCATAGCAGTAATG; and il-1β forward primer: TTCAGGCAGGCAGTATCACTC, reverse primer: CCACGGGAAAGACACAGGTAG genes. The mRNA levels were normalized to the expression levels of the control GAPDH forward primer: AGGTCGGTGTGAACGGATTTG, reverse primer: TGTAGACCATGTAGTTGAGGTCA gen. For the data analysis, the ΔΔCt method was used to determine the fold change of all of the target genes in each sample with 95 % confidence. The qRT-PCR reaction for each gene was performed in duplicate, and each experiment was repeated at least three times. The PCR cycles were performed according to the manufacturer's instructions.

1.9 Contractile activity of ileal longitudinal smooth muscle

At the end of the experimental period, on day 8, the animals were euthanized in a CO_2 chamber, and then segments of terminal ileum (1 cm long) were removed from C57BL/6 (WT) and $P2X7^{-/-}$ mice (2-3 months old). The longitudinal muscle strips were vertically mounted in a 5-ml organ bath filled with Krebs-Ringer solution (in mM; NaCl 118.3; KCl 4.7; $CaCl_2$ 2.5; $MgSO_2$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25; glucose 11.1, pH 7,4) which was bubbled with carbogen (5% CO_2 and 95% O_2) at 37 °C. The tissues were equilibrated under a tension of 1 g (~ 10 mN) for 30 min until baseline tension was stable. At the end of the equilibration period, to test tissue viability, the ileum was contracted by adding KCl (100 mM) for ~5 min and 10 μM carbachol. Then the tissue was washed twice with the warm aerated Krebs solution at an interval of 15 min. Dose-response curves to acetylcholine (ACh) (0.01 – 30 μM) were constructed. The contractile responses were measured using an isometric force transducer and expressed as mN. Data were analyzed by nonlinear regression to estimate efficacy (E_{max}).

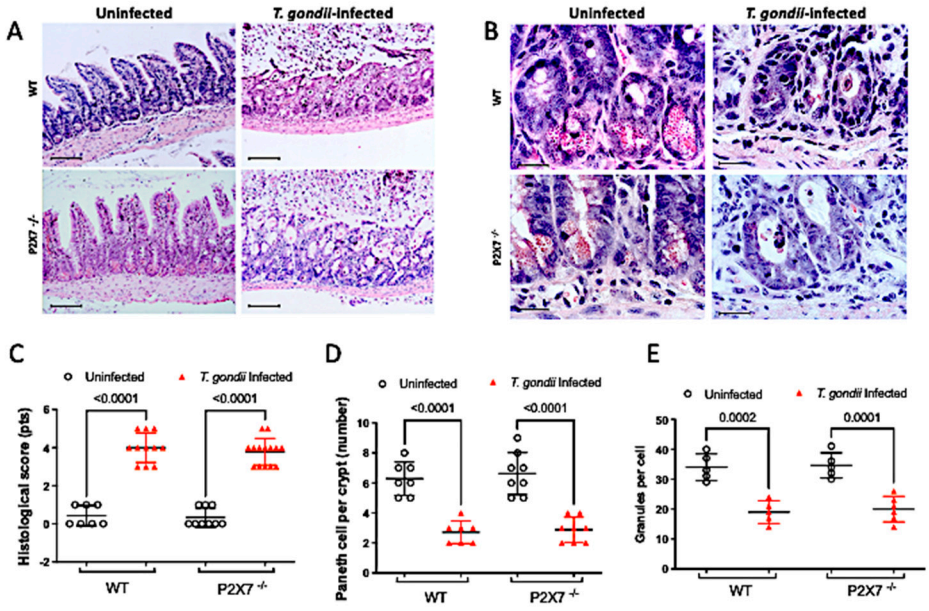
1.10 Fecal microbiota composition

Twenty-four samples (six per each experimental group) had their microbiome accessed from metabarcode analysis based on 16S rRNA, V3-V4 region [1]. The samples were extracted with the PureLink Microbiome DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The quality and quantity of the DNA were evaluated using a Nanodrop 2000 UV-vis Spectrophotometer (Thermo Scientific). Libraries were constructed using Nextera XT DNA Library Preparation kit (Illumina, California, U.S.A.) following the standard protocol. The quality of the libraries was measured using 2100 Bioanalyzer

Instrument. A pool was made with an aliquot of each sample. The samples were sequenced in the MiSeq System (Illumina, California, U.S.A.) and demultiplexed using by Illumina system.
The quality of the sequences was verified using MultiQC [2] . Samples were analyzed using Qiime2 (2019.7) platform (Boylen et al., 2019). The samples were denoised using the deblur extension, all amplicon sequence variants (ASVs) were aligned with Mafft, and the phylogeny was constructed using FastTree [3, 4]. Taxonomical annotation was made using vsearch and based on Silva v.123 database [5]. Barplots were constructed using the barplot extension, the alpha diversity was calculated based on Faith's Phylogenetic Diversity using alpha-group-significance extension, and the beta diversity was calculated based on weighted unifrac using the beta-group-significance extension [6, 7].

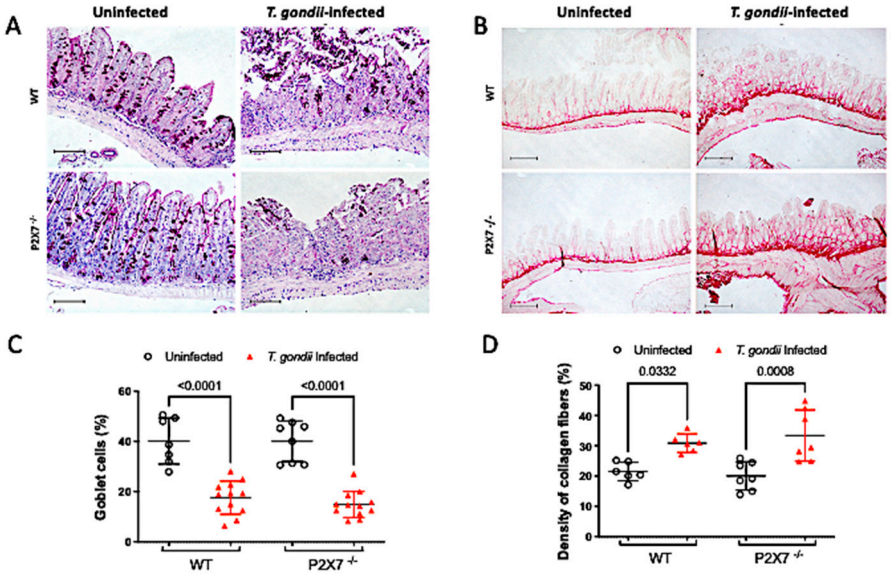
2. Supplementary Results:

2.1 Supplementary Figure S1



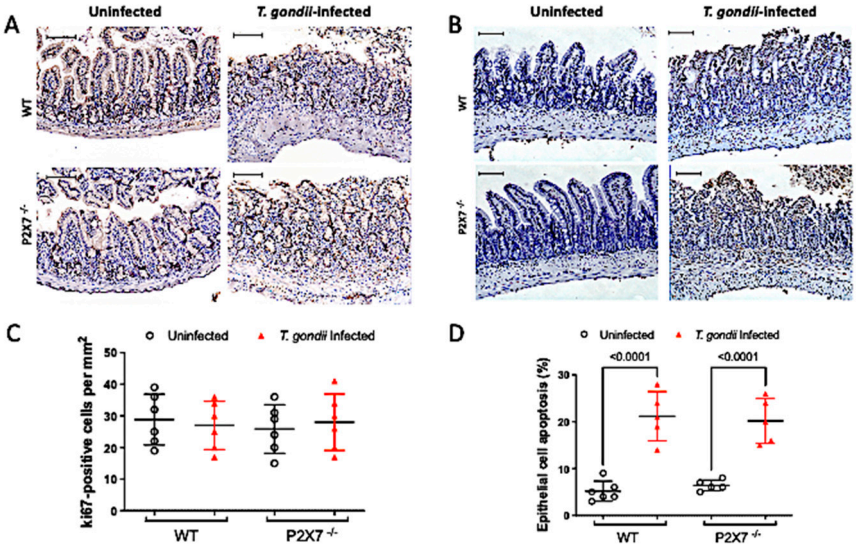
Supplementary Figure S1. *T. gondii* infection induces severe ileal inflammation and damage, with a significantly greater extension among the P2X7-/- mice. Histopathological analysis by hematoxylin and eosin (HE) staining of the terminal ileum shows severe inflammatory changes and tissue damage initiated by *T. gondii*-infection, but apparently not affected by the expression of the P2X7 (A, B). The density of Paneth cells and their granules showed a significant reduction in infected animals, but no change was attributed to the P2X7 expression (D, E). The scale bars represent 50μm. Data represent the means with SD of 7 to 12 animals per group. The analysis was performed by ANOVA, in which the post hoc Tukey test was used for multiple comparisons. Significant differences are presented.

2.1 Supplementary Figure S2



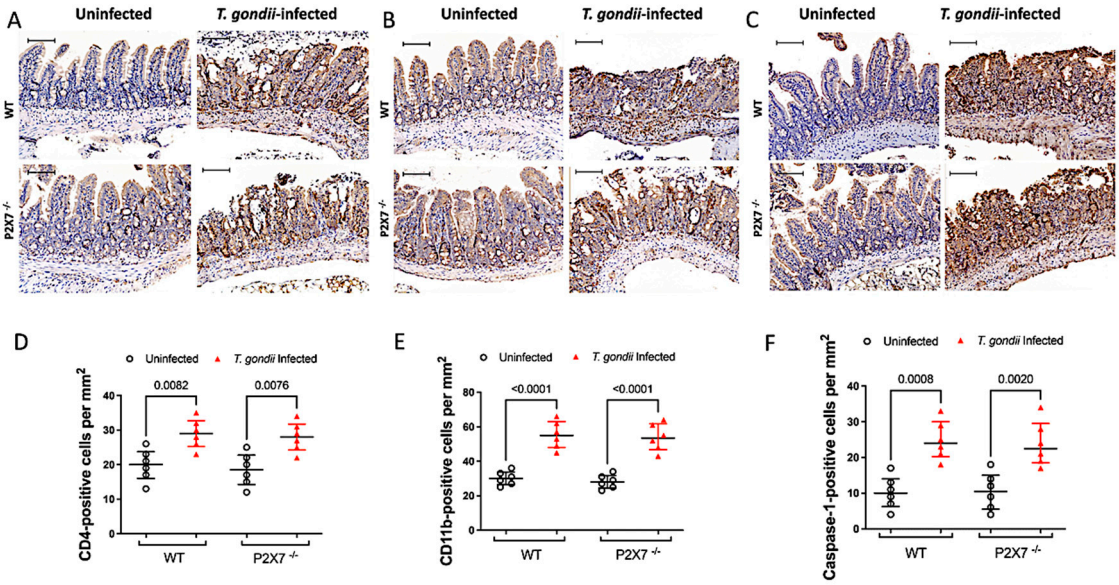
Supplementary Figure S2. *T. gondii* infection results in intense damage to specialized epithelial cells and deposition of collagen fibers in the ileum. Histopathological analysis revealed the increased loss of mucous-producing goblet cells labeled with periodic acid-Schiff (PAS) (A) and accumulation of collagen fibers labeled with picrosirius red dye (B) in *T. gondii*-infected mice. However, no difference was detected comparing WT with P2X7-/- mice (D, E). The scale bars represent 50μm. Data represent the means with SD of 6 to 12 animals per group. The analysis was performed by ANOVA, in which the post hoc Tukey test was used for multiple comparisons. Significant differences are presented.

2.1 Supplementary Figure S3



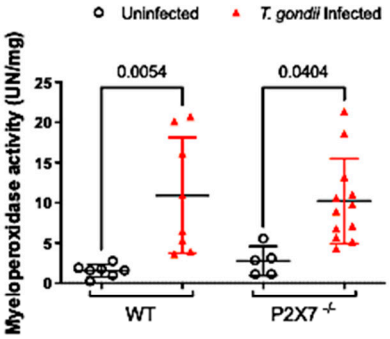
Supplementary Figure S3. *T. gondii* infection is characterized by intense ileitis with marked cell loss. Regenerative mechanisms may not be sufficient in the acute phase of the infection, and the expression of the ki67-proliferation cell marker did not show any difference among the groups (A, C). Nevertheless, apoptotic cells detected using a TUNEL assay showed a marked increase in *T. gondii*-induced mice, but no difference was observed regarding the P2X7 expression (B, D). Values are the means with SD of 5 to 6 animals per group. The analysis was performed by ANOVA, in which the post hoc Tukey test was used for multiple comparisons. Significant differences are presented.

2.1 Supplementary Figure S4



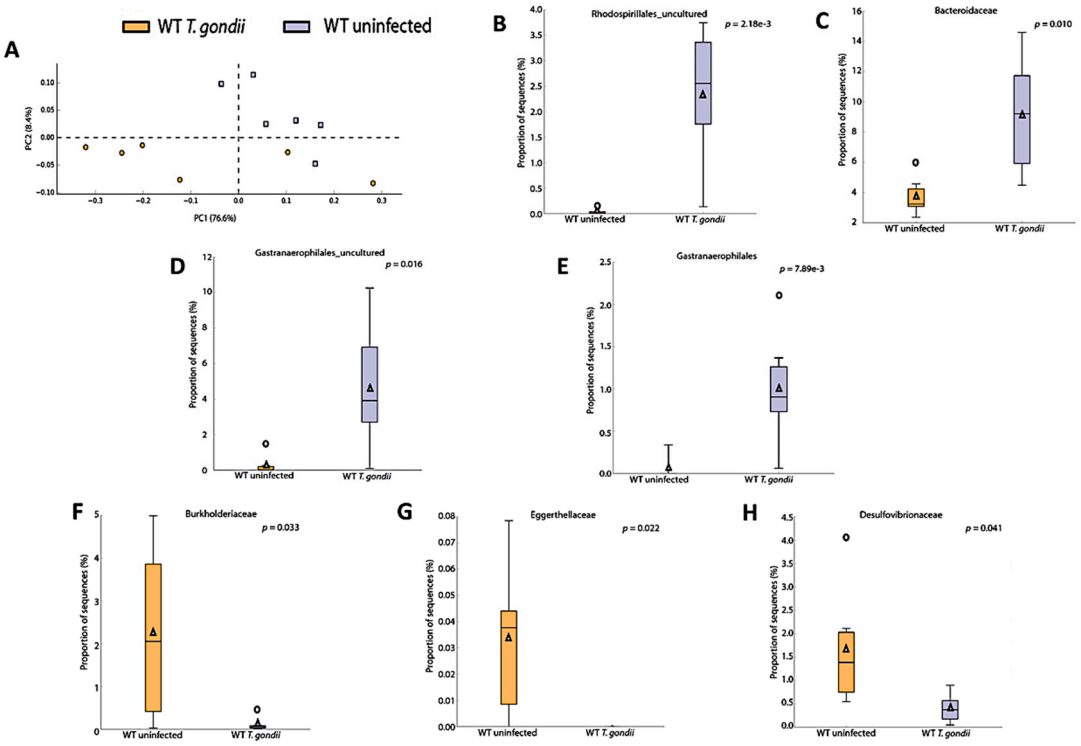
Supplementary Figure S4. *T. gondii*-induced ileitis is characterized by a marked inflammatory cell accumulation and activation in the intestinal mucosa. The expression of CD4- and CD11b-positive cells, representing mononuclear immune-active cells, and caspase-1, a critical enzyme for activating IL-1 beta, for example (A,B,C), were significantly increased in infected mice, but no difference was detected regarding the expression of the P2X7 (D, E, F). Values are the means with SD of 5 to 6 animals per group. The analysis was performed by ANOVA, in which the post hoc Tukey test was used for multiple comparisons. Significant differences are presented.

2.1 Supplementary Figure S5



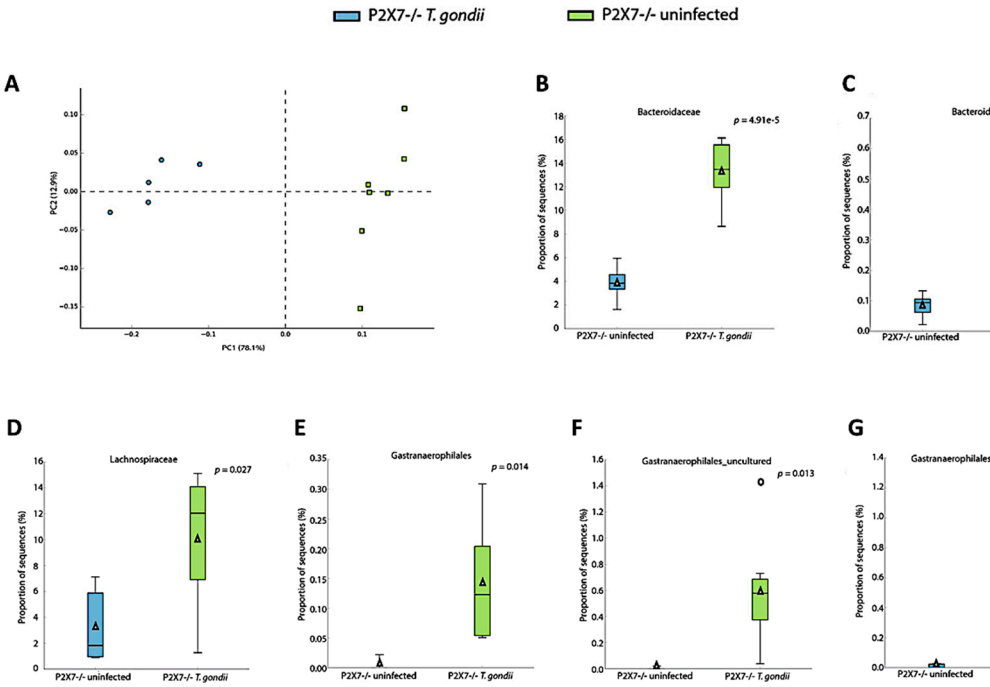
Supplementary Figure S5. *T. gondii*-induced ileitis is characterized by increased myeloperoxidase activity (MPO) in the affected tissue. However, no significant difference was observed regarding the expression of the P2X7. Values are the means with SD of 5 to 12 animals per group. The analysis was performed by ANOVA, in which the post hoc Tukey test was used for multiple comparisons. All statistical values are presented.

2.1 Supplementary Figure S6



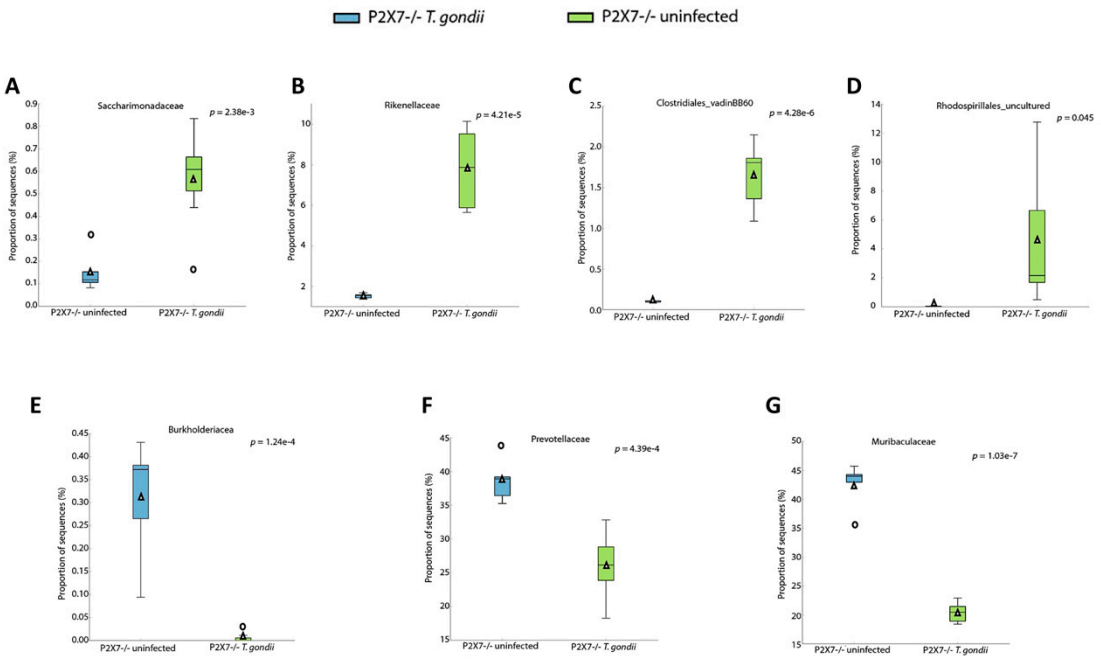
Supplementary Figure S6. *T. gondii*-induced ileitis is associated with changes in the gut microbiota of the WT mice. The Principal Component Analysis (PCA) shows the differences between the WT *T. gondii* and WT uninfected groups (A). The box plots show the distribution in the proportion of operational taxonomic units (%). WT *T. gondii* infection induced an increase in Rhodospirillales (B), Bacteroidaceae (C), and Gastranaerophilales (D, E), and a reduction in Burkholderiaceae (F), Eggerthellaceae (G), and Desulfovibrionaceae (H), compared with WT uninfected. The boxes represent the interquartile ranges (IQR) of 6 to 7 animals per group. The median value is shown as a line within the box and the mean as a triangle. Whiskers extend to the most extreme value. Outliers are shown as a circle. The *p*-value analysis was performed by Kruskal-Wallis, in which multiple comparisons were carried out using Dunn's post hoc test. Considering the *p*-value of 0.05, the variations shown are significant.

2.1 Supplementary Figure S7



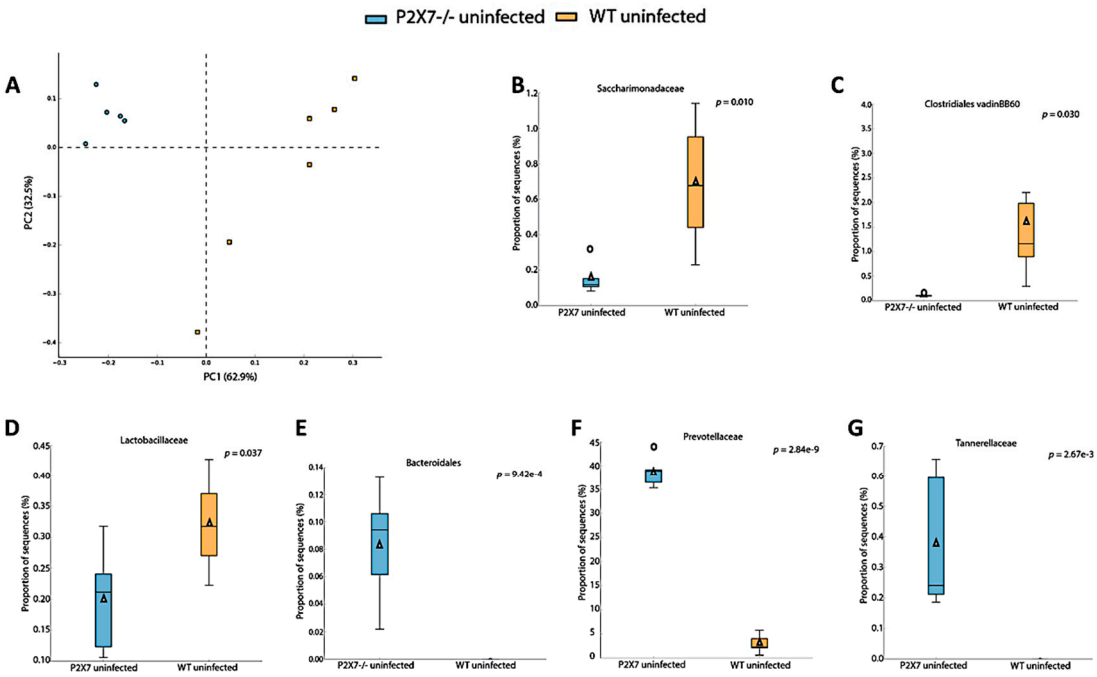
Supplementary Figure S7. *T. gondii*-induced ileitis is associated with changes in the gut microbiota of the P2X7-/- mice. The Principal Component Analysis (PCA) shows a clear separation between the P2X7-/- *T. gondii* and P2X7-/- uninfected groups (A). The box plots show the distribution in the proportion of operational taxonomic units (%). P2X7-/- T. gondii infection induced increases in Bacteroidaceae (B), Bacteroidales (C), Lachnospiraceae (D), and Gastranaerophilales (E, F, G), compared with P2X7-/- uninfected. The boxes represent the interquartile ranges (IQR) of 6 to 7 animals per group. The median value is shown as a line within the box and the mean as a triangle. Whiskers extend to the most extreme value. Outliers are shown as a circle. The *p*-value analysis was performed by Kruskal-Wallis, in which multiple comparisons were carried out using Dunn's post hoc test. Considering the *p*-value of 0.05, the variations shown are significant.

2.1 Supplementary Figure S8



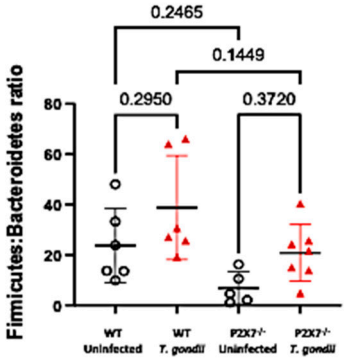
Supplementary Figure S8. *T. gondii*-induced ileitis is associated with changes in the gut microbiota of the P2X7-/- mice. The box plots show the distribution in the proportion of operational taxonomic units (%). P2X7-/- *T. gondii* infection induced increases in Saccharimonadaceae (A), Rikenellaceae (B), Clostridiales vadin (C), Rhodospirillales (D), and reductions in Burkholderiacea (E), Prevotellaceae (F), and Muribaculaceae (G) compared with P2X7-/- uninfected. The boxes represent the interquartile ranges (IQR) of 6 to 7 animals per group. The median value is shown as a line within the box and the mean as a triangle. Whiskers extend to the most extreme value. Outliers are shown as a circle. The *p*-value analysis was performed by Kruskal-Wallis, in which multiple comparisons were carried out using Dunn's post hoc test. Considering the *p*-value of 0.05, the variations shown are significant.

2.1 Supplementary Figure S9



Supplementary Figure S9. The P2X7 receptor modulates the gut microbiota in mice. The Principal Component Analysis (PCA) shows a clear separation between P2X7-/- uninfected and WT uninfected mice (A). The box plots show the distribution in the proportion of operational taxonomic units (%). P2X7-/- uninfected is associated with a reduction in Saccharimonadaceae (B), Clostridiales vadin (C), and Lactobacillaceae (D), and increases in Bacteroidales (E), Prevotellaceae (F), and Tannerelaceae (G), compared with WT uninfected. The boxes indicate the interquartile ranges (IQR) of 6 to 7 animals per group. The median value is shown as a line within the box and the mean as a triangle. Whiskers extend to the most extreme value. Outliers are shown as a circle. The *p*-value analysis was performed by Kruskal-Wallis, in which multiple comparisons were carried out using Dunn's post hoc test. Considering the *p*-value of 0.05, the variations shown are significant.

2.1 Supplementary Figure S10



Supplementary Figure S10. Effect of P2X7 on microbiota composition considering the Firmicutes: Bacteroidetes ratio of fecal samples in *T. gondii*-induced ileitis. No significant changes were detected among the experimental groups. Values are the means with SD of 5 to 7 animals per group. The analysis was performed by ANOVA, in which the post hoc Tukey test was used for multiple comparisons. All statistical values are presented.

3. References

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DISCUSSÃO

O receptor P2X7 é expresso por diferentes tipos celulares da mucosa intestinal, incluindo células epiteliais intestinais (CAMPOS *et al.*, 2012). Em 2009, Welter-Stahl *et al.* demonstrou que o tratamento de células epiteliais humanas com IFN-γ regula positivamente a expressão e a funcionalidade do receptor P2X7. No intestino delgado, o epitélio é menos responsivo ao ATP em comparação com as células epiteliais derivadas do cólon. Entretanto, o receptor P2X7 é regulado positivamente em células epiteliais do intestino delgado após o tratamento com IFN-γ (CAMPOS *et al.*, 2012). O tratamento de macrófagos humanos com TNF-α, induziu um aumento na expressão do receptor P2X7. Além disso, foi identificado que TNF-α atua em sinergia com IFN-γ potencializando o efeito sobre o receptor P2X7 em macrófagos (HUMPHERYS & DUBYAK, 1998).

No contexto infeccioso, foi identificado que após a infecção de macrófagos com *Leishmania amazonensis (L. amazonensis)* ocorreu um aumento significativo na expressão do receptor P2X7, o que torna a célula infectada mais suscetível à apoptose (CHAVES *et al.*, 2009). Em 2014, Chaves *et al*. demonstrou que o receptor P2X7 elimina a infecção por *L. amazonensis* após o tratamento com ATP. Em modelo *in vivo* de infecção por *Plasmodium chabaudi*, foi observado que animais $P2X7^{-/-}$ apresentaram maior suscetibilidade à infecção, fato correlacionado à diferenciação prejudicada de células Th1 (SALLES *et al*., 2017).

O receptor P2X7 também desempenha papel importante no controle da infecção por *T. gondii*. A ativação do receptor P2X7 em macrófagos infectados leva a eliminação do parasita pela produção de ROS e acidificação do vacúolo parasitóforo (CORREA *et al*., 2010; LEES *et al.*, 2010). Em células epiteliais intestinais, o receptor P2X7 induz a produção de citocinas e quimiocinas que contribuem para a resposta imunológica do hospedeiro contra a infecção por *T. gondii* (HUANG *et al*., 2017).

O presente trabalho demonstrou que a infecção oral por cistos de *T. gondii* induziu um aumento significativo na expressão do receptor P2X7 no íleo de camundongos. Ademais, observou-se que animais $P2X7^{-/-}$ são mais suscetíveis à infecção por *T. gondii*. Além disso, foi visto que o receptor P2X7 é crucial para controlar a extensão da lesão na mucosa intestinal desencadeada pela infecção por *T. gondii* e a carga parasitária no íleo. Paralelamente, foi identificado que os níveis de citocinas pró-inflamatórias locais e sistêmicas como IL-1β, IL-6 e IFN-γ apresentaram-se menores nos animais $P2X7^{-/-}$ infectados em comparação com os níveis observados em animais selvagens infectados.

O aumento significativo da carga parasitária pode estar relacionada diretamente com a produção prejudicada de moléculas inflamatórios nos animais deficientes para o receptor P2X7. Por isso, é possível inferir que o receptor P2X7 participa da resposta imunológica promovendo a produção de citocinas pro-inflamatórias que atuam no controle da carga parasitaria. Em 2017, Correa *et al*. demonstrou que animais P2X7$^{-/-}$ infectados com taquizoítas da cepa RH pela via intraperitoneal não apresentaram aumento dos níveis de IL-12, IL-1β, IFN-γ e TNF-α no lavado peritoneal. Além disso, foi demonstrado que animais P2X7$^{-/-}$ apresentam maior suscetibilidade à infecção por *T. gondii*.

No presente trabalho, a proliferação descontrolada do parasita, observada no íleo dos animais P2X7$^{-/-}$, pode ter contribuído para a disseminação do *T. gondii* em nível sistêmico, pois observou-se altos níveis de ALT e AST no soro dos animais P2X7$^{-/-}$ infectados. Esse achado nos permite afirmar que a infecção pelo *T. gondii* em animais P2X7$^{-/-}$ induziu um comprometimento hepático maior em comparação com os animais selvagens infectados. Em 2017, Correa *et al*. demonstrou que animais P2X7$^{-/-}$ infectados com taquizoítas da cepa RH pela via intraperitoneal apresentam aumento nos níveis sorológicos de ALT e dano hepático acentuado.

O bloqueio ou deleção de receptores P2X7 está associado a redução do dano tecidual e dos sintomas em modelos de inflamação intestinal. A expressão do receptor P2X7 em neurônios entéricos está envolvida na morte dessas células e mudanças na motilidade (JOOSS *et al.*, 2023). A infecção por *T. gondii* desencadeia alterações no sistema nervoso entérico (FEREZIN *et al.*, 2017) e pode modular a motilidade intestinal (MACHADO *et al.*, 2020). No presente estudo demonstramos que o receptor P2X7 possui papel na motilidade do íleo durante a infecção por *T. gondii*, corroborando com os resultados descritos na literatura.

Os inflamassomas são complexos multiproteicos citosólicos que respondem a moléculas microbianas e regulam a resposta imune inata contra patógenos invasores. Em geral, proteínas sensoras como NLRP3 ou AIM2 detectam PAMPs ou DAMPS no citoplasma para iniciar a oligomerização, facilitando o recrutamento da proteína adaptadora ASC. A plataforma do inflamassoma leva a dimerização da caspase-1, ativando assim a sua função protease e, subsequentemente desencadeia a liberação de IL-1β e IL-18 em suas formas maduras. Diferentes fatores, incluindo fluxo de íons, ATP e ROS foram associados a ativação de NLPR3 pela via canônica. Evidências sugerem que *T. gondii* pode ativar os inflamassomas NLRP1, NLRP3 e AIM2 (WANG *et al*., 2020).

O controle da infecção por *T. gondii* via P2X7 depende do inflamassoma canônico, mas não de caspase-11. A eliminação do parasita via receptor P2X7 e ativação do inflamassoma

foi dependente da geração de ROS. O tratamento com ATP extracelular aumentou a secreção de IL-1β em macrófagos infectados e esse efeito foi dependente da via canônica do inflamassoma NLRP3 (MOREIRA-SOUZA *et al.*, 2017).

No presente estudo foi visto que a infecção por *T. gondii* em animais selvagens regula positivamente a expressão de NLRP3, IL-1β e caspase-1. Esses resultados nos permitem afirmar que a ativação do receptor P2X7 está diretamente associada com a ativação da resposta imunológica inata frente ao desafio infeccioso. A deficiência no receptor P2X7 compromete a habilidade de produzir e secretar citocinas pró-inflamatórias maduras, fato que culmina em descontrole da infecção pelo parasita. Esses resultados corroboram com o descrito por Quan *et al.*, 2018 que demonstrou que em células epiteliais do intestino delgado, a secreção de IL-1β dependente de NLRP3 é mediada pela ativação do receptor P2X7 em resposta à infecção por *T. gondii*, controlando a proliferação do parasita.

O NO possui efeitos microbicidas potentes em diversos microrganismos patogênicos intracelulares, como o *T. gondii*. O NO atravessa as membranas da célula infectada e interage diretamente com o parasita no interior dos vacúolos parasitóforos (YAROVINSKY, 2014). Animais deficientes para a enzima iNOS apresentam maior letalidade frente à infecção com *T. gondii*, fato associado ao aumento da carga parasitária (SASAI *et al*., 2018). Similarmente ao descrito na literatura, no presente estudo, identificamos um aumento nos níveis de NO no íleo dos animais infectados com *T. gondii,* sendo maior em animais P2X7$^{-/-}$ infectados. Os altos níveis de NO em animais P2X7$^{-/-}$ infectados podem estar associados ao maior dano na mucosa intestinal.

A disbiose da microbiota intestinal está associada a diversas doenças e com resposta inflamatória desregulada, incluindo a produção exacerbada de NO. Em 2021, Leclerc, *et al.*, demostrou que a exposição a NO leva a uma mudança na diversidade e funcionalidade da microbiota intestinal. A infecção oral por *T. gondii* resulta em disbiose bacteriana acentuada e patologia grave na porção distal do intestino delgado dependente de células T CD4 e INF-γ, contribuindo para o desenvolvimento da patologia no íleo. A produção de NO frente à infecção por *T. gondii* é importante para o controle da carga parasitária, entretanto está diretamente associado às modificações da composição da microbiota intestinal (WANG *et al*., 2020).

Alterações significativas na abundância relativa de bactérias cecais foram observadas em camundongos infectados por *T. gondii.* Identificou-se um aumento na abundância relativa de Proteobacterias, juntamente com bactérias nocivas, como Bilopha e Desulfovibrio (SHAO, 2020). No presente trabalho não foi identificado diferenças significativas na α-diversidade entre os grupos estudados. Observou-se somente uma tendência de diminuição nos animais com

toxoileíte. A indução de ileíte desencadeou mudanças estruturais na microbiota fecal. A microbiota apresentou-se composta principalmente pelos Filos Bacteroidetes e Firmicutes, seguidos pelos filos menos abundantes Proteobacteria e Epsilonbacteraeota e em nível de Filo não foram encontradas diferenças significativas entre os grupos estudados. Entretanto, no filo Bacteroidetes, foi observado aumento nas famílias Bacteridaceae e Rikenellaceae em resposta a infecção por *T. gondii*. Além disso, a toxoileíte reduziu a família Muribaculaceae e no filo Bacteroidetes. As alterações na microbiota intestinal desencadeadas após a infecção identificadas no presente estudo corroboram com os dados da literatura que sugerem que a disbiose intestinal pode ser induzida após a infecção por *T. gondii*.

Em 2022, Bernardazzi *et al*. demonstrou que há uma tendência maior na abundância relativa de populações da microbiota intestinal de camundongos $P2X7^{-/-}$. Algumas diferenças na abundância microbiana intestinal podem explicar a diferença na gravidade da ileíte induzida por *T. gondii* nos animais $P2X7^{-/-}$. As ordens Bacteroidales e Clostridiales foram relativamente mais abundantes nos animais $P2X7^{-/-}$ e aumentaram ainda mais após a infecção por *T. gondii*. Em conjunto, os resultados do presente estudo sugerem que a disbiose intestinal observada na infecção por *T. gondii* pode representar um fenômeno secundário, porém também pode preceder e favorecer a infecção.

CONCLUSÃO

Em conclusão, os resultados sugerem que a ativação do receptor P2X7 está envolvida na defesa do hospedeiro contra a infecção oral por *T. gondii*, pela limitação da patologia intestinal, controle da carga parasitária e regulação da resposta imunológica local e sistêmica. Além disso, foi possível observar que a via do receptor P2X7 também pode moldar a microbiota intestinal e determinar direta ou indiretamente o resultado do desafio infeccioso.

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APÊNDICES

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Sulfate-reducing bacteria stimulate gut immune responses and contribute to inflammation in experimental colitis

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ABSTRACT

The intestinal microbiota is critical for mammalian immune system development and homeostasis. Sulfate-reducing bacteria (SRB) are part of the normal gut microbiota, but their increased levels may contribute to colitis development, likely in association with hydrogen sulfide (H_2S) production. Here, we investigated the effects of SRB in the gut immune response in germ-free mice, and in experimental colitis. After 7 days of colonization with *Desulfovibrio indonesiensis* or with a human SRB consortium (from patients with colitis), germ-free mice exhibited alterations in the colonic architecture, with increased cell infiltration in the lamina propria. SRB colonization upregulated the Th17 and Treg profiles of cytokine production/cell activation, in T cells from mesenteric lymph nodes. These alterations were more pronounced in mice colonized with the human SRB consortium, although *D. indonesiensis* colonization produced higher levels of H_2S. Importantly, the colon of C57BL/6 mice with colitis induced by TNBS or oxazolone had increased SRB colonization, and the administration of *D. indonesiensis* to mice with TNBS-induced colitis clearly exacerbated the alterations in colonic architecture observed in the established disease, and also increased mouse weight loss. We conclude that SRB contribute to immune response activation in the gut and play an important role in colitis development.

1. Introduction

In mammals, the microbial community regulates important host metabolic and physiological functions, playing key roles in the development and homeostasis of the host immune system. The microbiota is crucial for the development and differentiation of both local and systemic components of innate and adaptive immunity, through its interaction with the mucosal immune system [1]. In particular, the gut microbiota appears to direct the organization and maturation of lymphoid tissues [2]. In the gut, the recognition of microbial compounds is essential for host T cell maturation, and for these cells to be recruited, as well as induced to differentiate and eventually reside in the gut [3]. Additionally, the recognition of microbial components drives the shift to high affinity IgA in the Peyer's Patch and is essential for B-cell mediated immune responses [4].

The importance of the microbiota for the development of mucosal immune responses is particularly evident in germ-free mice, which lack commensal bacteria and, thus, exhibit extensive defects in the gut-associated lymphoid system and in antibody production [5]. Germ-free mice are, therefore, more susceptible to infections by certain pathogens [6], even when compared with specific pathogen-free (SPF) mice [7].

Changes in the composition of the gut microbiota can contribute to the development of different pathological conditions, including obesity, autoimmune diseases and colitis [8–10]. Altered immune responses and their relationship with commensal microbiota that inhabit gastrointestinal tract are key aspects of the development of inflammatory bowel diseases (IBD) - namely Crohn's disease (CD) and ulcerative colitis (UC) - characterized by excessive (i.e., non-protective) inflammation [11,12]. Both systemic and local immune responses against mucosal bacteria appear increased in IBD [13–15]. Also, in fecal samples

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REVIEW

Damage-associated molecular patterns in inflammatory bowel disease: From biomarkers to therapeutic targets

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Abstract

The chronic inflammatory process underlying inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), derives from the interplay of several components in a genetically susceptible host. These components include environmental elements and gut microbiota a dysbiosis. For decades, immune abnormalities have been investigated as critically important in IBD pathogenesis, and attempts to develop effective therapies have predominantly targeted the immune system. Nevertheless, immune events represent only one of the constituents contributing to IBD pathogenesis within the context of the complex cellular and molecular network underlying chronic intestinal inflammation. These factors need to be appreciated within the milieu of non-immune components. Damage-associated molecular patterns (DAMPs), which are essentially endogenous stress proteins expressed or released as a result of cell or tissue damage, have been shown to act as direct pro-inflammatory mediators. Excessive or persistent signalling mediated by such molecules can underlie several chronic inflammatory disorders, including IBD. The release of endogenous DAMPs amplifies the inflammatory response driven by immune and non-immune cells and

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Schistosoma mansoni Coinfection Attenuates Murine *Toxoplasma gondii*-Induced Crohn's-Like Ileitis by Preserving the Epithelial Barrier and Downregulating the Inflammatory Response

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Background and aims: Mice orally infected with *T. gondii* develop Crohn's disease (CD)-like enteritis associated with severe mucosal damage and a systemic inflammatory response, resulting in high morbidity and mortality. Previously, helminthic infections have shown therapeutic potential in experimental colitis. However, the role of *S. mansoni* in *T. gondii*-induced CD-like enteritis has not been elucidated. Our study investigated the mechanisms underlying *T. gondii*-induced ileitis and the potential therapeutic effect of *S. mansoni* coinfection.

Methods: C57BL/6 mice were infected by subcutaneous injection of cercariae of the BH strain of *S. mansoni*, and 7–9 weeks later, they were orally infected with cysts of the ME49 strain of *T. gondii*. After euthanasia, the ileum was removed for histopathological analysis; staining for goblet cells; immunohistochemistry characterizing mononuclear cells, lysozyme expression, apoptotic cells, and intracellular pathway activation; and measuring gene expression levels by real-time PCR. Cytokine concentrations were measured in the serial serum samples and culture supernatants of the ileal explants, in addition to myeloperoxidase (MPO) activity.

Results: *T. gondii*-monoinfected mice presented dense inflammatory cell infiltrates and ulcerations in the terminal ileum, with abundant cell extrusion, apoptotic bodies, and necrosis; these effects were absent in *S. mansoni*-infected or coinfected animals. Coinfection preserved goblet cells and Paneth cells, remarkably depleted in *T. gondii*-infected mice. Densities of CD4- and CD11b-positive cells were increased in *T. gondii*- compared to *S. mansoni*-infected mice and controls. MPO was significantly increased among *T. gondii*-mice, while attenuated in coinfected animals.

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Small Intestine

Protective effect of adipose tissue–derived mesenchymal stromal cells in an experimental model of high-risk colonic anastomosis

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ABSTRACT

Background: Dehiscence of intestinal anastomosis results in high morbidity and mortality. The aim of this study was to investigate the effects of locally administered adipose tissue-derived mesenchymal stromal cells in a model of high-risk colonic anastomosis in rats.

Methods: Seven days after induction of colitis with 2,4,6-trinitrobenzene sulfonic acid, Wistar rats were submitted to a transection of the descending colon followed by end-to-end anastomosis and were then treated with 2×10^6 adipose tissue-derived mesenchymal stromal cells (from the preperitoneal fat) or an acellular culture solution instilled onto the surface of the anastomosis. At day 14, after macroscopic survey of the abdominal cavity, the anastomotic area was submitted to histologic and immunohistochemical analysis, evaluation of myeloperoxidase activity, fibrosis, epithelial integrity, NF-κ B activation, expression of inflammatory cytokines, and extracellular matrix-related genes.

Results: Anastomotic leakage and mortality associated with high-risk anastomosis decreased with treatment with adipose tissue-derived mesenchymal stromal cells ($P < .03$). Application of adipose tissue-derived mesenchymal stromal cells resulted in lower histologic scores ($P = .011$), decreased deposition of collagen fibers ($P = .003$), preservation of goblet cells ($P = .033$), decreased myeloperoxidase activity ($P = .012$), decreased accumulation of CD4+ T-cells ($P = .014$) and macrophages ($P = .011$) in the lamina propria, a decrease in the number of apoptotic cells ($P = .008$), and the activation of NF-κ B ($P = .036$). Overexpression of IL-17, TNF-α, IFN-γ, and metalloproteinases in the acellular culture solution–treated, high-risk anastomosis group decreased ($P < .05$) to near normal values with adipose tissue-derived mesenchymal stromal cells treatment.

Conclusion: Improvements in outcomes of a high-risk colonic anastomosis with adipose tissue-derived mesenchymal stromal cells therapy reflect the immunomodulatory activity and healing effect of these cells, even after just topical administration and reinforces their use in future translational research.

Introduction

Among the postoperative complications in colorectal operations, anastomotic dehiscence represents a serious problem to the patient because of its potential consequences, such as fecal peritonitis and sepsis.[1] It has been estimated that about one third of deaths related to anastomotic dehiscence can be attributed to the

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ORIGINAL ARTICLE

Observational Study

Serum 1,3-beta-D-glucan as a noninvasive test to predict histologic activity in patients with inflammatory bowel disease

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Author contributions: Farias e Silva K and Carneiro AJV participated in the conception and design of the study, the acquisition, analysis, and interpretation of data, and the drafting of the manuscript; Nanini HF, Cascabulho CM, Rosas SLB, and Santana PT participated in the acquisition, analysis, and interpretation of data; Anaissie E conceptually proposed the idea of gut inflammation and high 1,3-Beta-D-glucan levels and critically revised the manuscript; Nucci M and de Souza HSP participated in the conception and design of the study, obtained funding, analyzed and interpreted the data, and critically revised the manuscript for important intellectual content; all the authors gave final approval of the submitted version of the manuscript.

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Abstract

BACKGROUND

1,3-beta-D-glucan (BG) is a ubiquitous cell wall component of gut micro-organisms. We hypothesized that the serum levels of BG could reflect active intestinal inflammation in patients with inflammatory bowel disease.

AIM

To determine whether the serum BG concentrations correlate with intestinal inflammation.

METHODS

A prospective observational study was performed in a tertiary referral center, from 2016 to 2019, in which serum BG was determined in 115 patients with Crohn's disease (CD), 51 with ulcerative colitis (UC), and 82 controls using a photometric detection kit. Inflammatory activity was determined by ileocolonoscopy, histopathology, magnetic resonance enterography, and biomarkers, including fecal calprotectin (FC), C-reactive protein, and a panel of cytokines. The ability of BG to detect active *vs* inactive disease was assessed using the area under the receiver operating characteristic curve. In subgroup analysis, serial BG was used to assess the response to therapeutic interventions.

molecules

MDPI

Article

Bioactive Compounds from *Pale Ale* Beer Powder Attenuate Experimental Colitis in BALB/c Mice

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Abstract: Phenolic compounds (PCs) present in foods are associated with a decreased risk of developing inflammatory diseases. The aim of this study was to extract and characterize PCs from craft beer powder and evaluate their potential benefits in an experimental model of inflammatory bowel disease (IBD). PCs were extracted and quantified from pure beer samples. BALB/c mice received either the beer phenolic extract (BPE) or beer powder fortified with phenolic extract (BPFPE) of PCs daily for 20 days by gavage. Colon samples were collected for histopathological and immunohistochemical analyses. Dextran sodium sulfate (DSS)-induced mice lost more weight, had reduced colon length, and developed more inflammatory changes compared with DSS-induced mice treated with either BPE or BPFPE. In addition, in DSS-induced mice, the densities of CD4- and CD11b-positive cells, apoptotic rates, and activation of NF-κB and p-ERK1/2 MAPK intracellular signaling pathways were higher in those treated with BPE and BPFPE than in those not treated. Pretreatment with the phenolic extract and BPFPE remarkably attenuated DSS-induced colitis. The protective effect of PCs supports further investigation and development of therapies for human IBD.

Keywords: bioactive compounds; experimental colitis; *Pale Ale* beer powder; phenolic acids

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1. Introduction

Beer is an alcoholic drink produced by the brewing and fermentation of starches, mainly derived from malted barley, through the action of yeast, with the addition of hops. Special beers, also known as craft beers, have different characteristics compared with those produced on a large scale. The quality of these products is influenced by several variables, such as the quality of the raw material, malting type, wort preparation method, type of hops, type and quality of yeasts, fermentation time, maturation, and the use of processes such as pasteurization and filtration [1]. In addition to their worldwide acceptability, beers also have a growing appeal for functional properties because of the

including DM, HTN, ESRD, CAD, CHF, Charlson Comorbidity Index and other variables significant in pre-match analysis. Logistic regression was used for multivariate analysis (SAS 9.3). **Results** During the study period, 5,254 patients were discharged with a primary diagnosis of SM. We noted an annual percent change (APC) of +27.8% in total SM cases hospitalized per 100,000 pts during the study period. Hospitalization costs for SM increased similarly with APC of +6.6% whereas LOS and mortality decreased with APC of -0.7% and -8.7%, respectively (Table 1). Among the 5,254 pts, 28.6% (1169) pts underwent PEx. Pts in the PEx arm had lower comorbidities at baseline as defined by the Charlson Comorbidity Index (0.2±0.5 vs 0.3±0.6, p <0.001). Despite this, PEx were more likely to develop severe complications compared with the non-surgical arm. These complications included bowel obstruction (17.6%, 8.6%, P<0.001), bowel ischemia (5.6, 3.2%, P=0.0001), ileus (16.4, 6.7%, P<0.0001), malnutrition (7.1, 5.1%, P<0.001), respiratory failure (5.8, 2.8%, p<0.001) and a need for bowel resection (6.2, 1.4%, p<0.0001). After matching 1,148 pts in each group in a 1:1 ratio, no difference was noted with in-hospital mortality (2.2 v. 2.4%, p= 0.67). However, LOS (9.8±12.2 vs 7.1±11.2, p<0.0001) and hospitalization costs ($78,007± $121,804 vs $48,973 ± $98,688, p<0.0001) were higher for the PEx group. **Conclusion** SM related hospitalization rates are rising which might be related to increased frequency of imaging studies and better radiological recognition of this disease. SM poses a high health care burden with increasing hospitalization costs. Peritoneal surgical intervention is associated with higher rates of complications as well as greater healthcare utilization and burden. Further studies are needed to better delineate which pts would benefit most from operative interventions when imaging is consistent with SM.

Table 1: Annual Trends of SM related Hospitalization, Length of Stay, Hospitalization costs and Mortality

Year	2005	2006	2007	2008	2009	2010	2011	2012
Total SM cases	148	614	679	768	679	774	851	741
Total SM cases per 100000 admissions	1.9	7.6	8.5	9.4	8.7	9.9	10.6	10.2
Length of stay for SM cases (Days)	8.3	8.2	7.8	7.6	7.9	7.2	8.2	7.9
Hospitalization charges for SM cases (US Dollars)	42,820	42,444	44,916	54,521	56,459	55,656	69,618	66,761
Mortality of SM cases N (%)	5(3.4)	17(2.8)	19(2.8)	22(2.9)	13(1.9)	13(1.7)	9(1.1)	13(1.8)

Su1178

SERUM MMP-9 - A NOVEL BIOMARKER FOR PREDICTION OF CLINICAL RELAPSE IN PATIENTS WITH QUIESCENT SMALL BOWEL CROHN'S DISEASE

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Background Predicting relapse in Crohn's disease (CD) patients may allow an early optimization of treatment and possible reduction of subsequent complications. There is an increasing need for a novel and reliable noninvasive biomarkers for this purpose. Matrix metalloproteinase-9 (MMP-9) is a relatively new and promising marker of intestinal inflammation. We aimed to assess, for the first time, whether serum MMP-9 levels can predict clinical relapse in patients with quiescent small bowel CD. **Methods** This was a post-hoc analysis of patients with quiescent small bowel CD who were included in a prospective observational study and followed until clinical relapse or the end of the 24 months study. Clinical relapse was defined as an increase in CD activity index (CDAI) score of at least 70 points from baseline, or an intensification of therapy, as per physician global assessment based decision. The patients were followed with serial magnetic resonance enterography (MREs) and capsule endoscopies (CEs). Small bowel inflammation was quantified by Lewis score (LS) for CE. Magnetic resonance index of activity (MaRIA) score was calculated for MRE. C-reactive protein (CRP) and fecal calprotectin (FC) were obtained at baseline and at each follow-up visit. Serum MMP-9 levels in baseline blood samples were quantified by enzyme linked immunoabsorbent assay and the correlation of MMP-9 levels with subsequent clinical flare was investigated. **Results** Sixty one patients were enrolled. After exclusion of three blood samples due to technical causes, there were 58 analyzable samples from 58 CD patients (median age 29.5y, 43% female), of whom 16 have relapsed. Baseline MMP-9 was higher in patients who developed symptomatic relapse [median 661 ng/ml, 25-75 IQR (478.2-1441.3) vs. 525.5 ng/ml, (339-662.7), in relapsers versus non-relapsers, respectively, p = 0.01]. ROC analysis of serum MMP-9 levels showed a fair discriminatory accuracy with an AUC of 0.72 (95% CI: 0.56-0.88). MMP-9 concentrations ≥ 945 ng/ml yielded the optimal cut-off in terms of combined sensitivity of 44% and specificity of 100% for detecting relapse. Exploratory analysis including only the 13 patients who had CDAI change>70 points demonstrated an improved discriminatory accuracy with AUC of 0.76 (95% CI: 0.58-0.93). MMP-9 concentrations displayed a moderate and low correlation with baseline FC and LS respectively (r=0.46, p<0.0001; r=0.31, p<0.05). No correlation was found between serum MMP-9 and CRP levels or MaRIA score. **Conclusion** Serum MMP-9 may be a novel biomarker for prediction of relapse in CD patients with quiescent disease.

Su1179

PROTECTIVE EFFECT OF MESENCHYMAL STEM CELLS IN AN EXPERIMENTAL MODEL OF HIGH RISK INTESTINAL ANASTOMOSIS IN RATS

Valter Alvarenga, Hayandra F. Nanini, Natalia Bonfa, Beatriz P. Damasceno, Morgana T. Castelo-Branco, Wagner Baetas, Heitor S. de Souza, Alberto Schanaider

Background and aims: In the presence of inflammatory colitis, the dehiscence of intestinal anastomosis represents a severe complication with high morbidity and mortality. In this study, we investigated the potential healing effect of locally administered adipose tissue-derived mesenchymal stem cells (AT-MSCs) in a model of high-risk colonic anastomosis in rats. **Methods:** A 2,4,6-trinitrobenzene sulfonic acid (TNBS)-colitis was induced in specific pathogen-free male Wistar rats, and confirmed by colonoscopy. At day 7, a transversal section of the descending colon, 4 cm above peritoneal pelvic reflection, preserving the blood supply, and followed by end-to-end anastomosis was performed. Next, either $2X10^6$ AT-MSCs or cell culture medium was directly dripped over the entire external anastomosis surface. Two additional groups of animals not receiving TNBS, and submitted or not to the surgical procedure served as sham and control groups, respectively. Euthanasia was performed at day 14, when the abdominal cavity was surveyed and the area of anastomosis removed for further studies. Samples were analyzed for histologic scores, myeloperoxidase activity, fibrosis, inflammatory infiltrates, epithelial damage, inflammatory mediators in supernatants of explant cultures, and gene expression of molecules involved in wound healing, by real-time PCR. **Results**: Animals treated with AT-MSCs had fewer surgical complications including phlegmon, abscess, fistula, leakage and adhesions (80 versus 25%), compared with the group treated with conditioned cell culture medium. Only one anastomotic dehiscence occurred in the AT-MSCs treated group (6%), while a remarkably higher rate was observed in the cell culture medium-treated group (30%). Treatment with AT-MSCs resulted in a reduced area of inflammation (p=0.004), lower histopathologic score (p=0.011), reduction of myeloperoxidase activity (p=0.018), and collagen deposition (p= 0.003), and goblet cells preservation (p=0.033), compared to treatment with culture medium. The therapeutic effect of AT-MSCs was associated with reduction in the inflammatory cell infiltration of the lamina propria, epithelial apoptosis, the concentrations of Th1 and Th17 cytokines, and of the NF-kappa B activation. Moreover, abnormal expression of genes associated with extracellular matrix homeostasis was also observed in the model, probably reflecting tissue remodeling. **Conclusion**: Improvements in surgical outcomes in high-risk anastomosis after inflammatory colitis induction and observed with AT-MSCs therapy reflect the immunomodulatory activity and healing effect of these cells, even following topical administration, and reinforce their use in future translational research.

Su1180

THE EFFICACY OF TONSIL-DERIVED MESENCHYMAL STEM CELLS CONDITIONED MEDIUM IN CHRONIC COLITIS MODEL

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Background: Tonsil-derived Mesenchymal stem cells (TMSCs) obtained from tonsillectomy have many advantages such as a short doubling time, high differentiation capacity, and immune modulatory activity. In previous studies, intraperitoneal administration of TMSC in DSS- induced acute and chronic colitis animal models have shown results of improvement in disease activity index and down regulation of histological grading and pro-inflammatory cytokine expression levels. However, the TMSCs were not observed to migrate to the inflamed intestine *in vivo*. These results are presumed to be due to the paracrine effect of TMSCs. In this study, we tried to verify the therapeutic effect of TMSCs conditioned medium (TMSCs-CM) in the mouse model of DSS-induced chronic colitis. **Methods:** *In vitro*, immunosuppressive effects of TMSCs-CM were confirmed by splenocyte immunosuppression assay. C57BL/6 mouse splenocytes were stimulated with mitogen such as LPS or PMA/Ionomycine, and then cultured in TMSCs-CM or co-cultured with TMSC. After 24 hours, proliferation of splenocytes was measured using the cck-8 kit. *In vivo*, eight-week-old C57BL/6 mice were randomly assigned into 4 groups: normal, colitis, TMSC, and TMSC-CM groups. Chronic Colitis was induced by oral administration of 1.5% dextran sulfate sodium (DSS) for 5 days continuously followed by additional 5 days of plain water feeding and repeated with two more cycles (total 30 days). TMSC ($1x10^6$cells/500ul) and TMSC-CM (500ul) were administered via intraperitoneal injection for 4 and 12 times, respectively. The severity of colitis in each mouse was assessed by measuring their disease activity index (DAI), body weight change, colon length, histologic grading, and cytokine levels. **Results:** The splenocyte stimulated by LPS or PMA/Ionomycine showed decreased proliferation. When co-cultured with TMSC (3.18± 0.07 *vs.* 2.03 ± 0.12, mean ± standard error mean, control group *vs.*, $p = 0.0008$, ANOVA), and cultured in TMSC-CM (3.18 ± 0.07 *vs.* 1.81 ± 0.06, $p < 0.0001$). Proliferation was significantly reduced by the number of TMSC ($p = 0.0055$) and TMSC-CM concentration ($p = 0.0028$). In a chronic colitis animal model injected with TMSC [X4] or TMSC-CM [X12], reduction of DAI (3.25 ± 0.25 *vs.* 1.50 ± 0.28 *vs.* 1.50 ± 0.28, colitis *vs.* TMSC *vs.* TMSC-CM group, $p = 0.0038$), weight gain (1.65 ± 2.88 *vs.* 5.08 ± 3.31 *vs.* 6.00 ± 0.52, $P = 0.4562$, $p = 0.1888$, respectively), and recovery of colon length (61.4 ± 2.82 *vs.* 70.0 ± 2.74 *vs.* 72.60 ± 2.68, $p = 0.0602$, $p = 0.0205$, respectively.) was observed at day 30. **Conclusion:** In the DSS-induced chronic colitis animal model, the administration of TMSC as well as TMSC-CM showed almost the same effect on improvement of inflammation. Therefore, we suggest the use of TMSC-CM for IBD treatment utilizing the paracrine factors of TMSC without any cell transplantation.

model using colorectal biopsies from well-characterized UC patients and healthy controls (HC) to measure the impact of TOFA on inflammatory profiles with the ultimate goal of directing therapy. **Methods**: Patients with UC (n=8) or HC (n=8) underwent endoscopic collection of colorectal biopsies. Biopsies were placed directly into culture and remained unstimulated or were stimulated with phytohemagglutinin (PHA), a lectin that activates T-cells by binding to the T-cell receptor or Cytomix, a cocktail of proinflammatory cytokines (IL-6, IL-22, IFN-α) for 24 hours +/-10mM TOFA-citrate (Sigma-Aldrich, St. Louis, MO). Following incubation, explant supernatant was assayed for 30 cytokines, chemokines, and inflammatory mediators using the Mesoscale Discovery platform. Results, normalized for biopsy weight, were analyzed using a permutation test with a robust linear mixed model to reduce outlier weight. **Results:** Unstimulated UC explants produced greater levels of IL12-p40(p<0.05), TNF-β(p<0.01), IL-8(p<0.01), IP-10(p<0.01), MIP-1α(p<0.05), MIP-1β(p<0.05), TARC(p<0.05), IL12-p70(p<0.01), IL-13(p<0.01), IL-4(p<0.05), IL-6(p<0.05), TNF-α(p<0.05), IL-8(p<0.001) than explants from HC. Of these, TOFA inhibited expression of IP-10 (p<0.001) and TARC (p<0.01) from both HC and UC explants. Upon stimulation of UC explants with Cytomix, we observed a TOFA-related decrease in IL-17(p<0.01), Eotaxin-1(p<0.01), Eotaxin-3(p<0.01), IP-10(p<0.0001), MCP-1(p<0.0001), MIP-1α(p<0.05), MIP-1β(p<0.0001) and TARC(p<0.0001), as well as an increase in MDC(p<0.01). Similar results were obtained for Cytomix stimulated HC explants, except for TOFA inhibition of IL-17. TOFA also mitigated the induction of IL-17(p<0.01), IP-10(p<0.0001), IL-1α(p<0.01), MIP-1α(p<0.05), MIP-1β(p<0.0001), TNF-β(p<0.05) and IFN-γ(p<0.01) by PHA in UC explants and IP-10(p<0.0001), IL-1α(p<0.05), TNF-α(p<0.05), IFN-γ(p<0.01) and TARC(p<0.01) in HC explants. **Conclusions:** The JAK/STAT inhibitor TOFA inhibits UC-related inflammatory cytokines in untreated and PHA or Cytomix stimulated explants from UC patients and HC. This *ex vivo* colorectal explant model has great potential as a preclinical method to test new therapeutic agents. Future studies will include correlating results from this model with cytokines present in stool samples with the goal of developing rapid, non-invasive assays to assist physicians in selecting and monitoring therapeutic agents and efficacy.

Tu1783

BARRIER RESTITUTION EFFECTS OF EGCG DRIVEN IN PART BY ITS EFFECT ON IL-10 FAMILY CYTOKINES

Sauradeep Sarkar, Hassan M. Qazzaz, Gerald W. Dryden

Introduction: The anti-inflammatory properties of (-) epigallocatechin-3-gallate (EGCG), the major green tea polyphenol (GTP), have sparked considerable interest in its potential as a therapy for inflammatory bowel disease (IBD), including both Crohn's disease (CD) and ulcerative colitis (UC). In this study, we evaluated the effect of EGCG on IL-10 family members IL-10 and IL-22 in the epithelial Caco2 cell line in order to enhance our understanding of the role this compound plays in the treatment of IBD. IL-22 has demonstrated important effects on epithelial restitution and tight-junction protein (TJP) composition. Aim: Investigate the effect of EGCG on IL-10 family members IL-10 and IL-22 production by human Caco2 epithelial colon cells, evaluating its role in barrier restitution. Methods: CaCo-2 cells were used in this experiment. Untreated control cells were compared to cells treated with two concentrations of EGCG (5 or 10µg/ml), LPS, or both. Effects on production of IL-10 and IL-22 were measured by microbead ELISA and compared to effects on IL-12 family cytokines IL-12, IL-17A, and IL-23. RNA message for all cytokines was measured by real-time polymerase chain reaction. Specific claudins relevant to barrier function were measured by Western blot. Results: EGCG (5 or 10µg/ml) demonstrated striking effects on cytokine production and RNA message compared to basal production or LPS stimulated cells (Table1). EGCG significantly enhanced IL-10 and -22 production, while suppressing IL-12 family members. Interestingly, this pattern of cytokine modulation corresponded with a "tightening" effect as it modulated TJP composition in Caco-2 monolayers. LPS dramatically increased claudin-2 and down-regulated claudins-1 and 5. EGCG by itself enhanced occludin, ZO-1 and claudin-1 and -5, while decreasing claudin-2. EGCG co-administered with LPS reversed the decline in claudin-1 and -5, while suppressing claudin-2. These changes correlated with a reversal of LPS-induced changes in TER. Discussion: While initially considered to be pro-inflammatory and epithelial barrier disrupting, IL-22 has now been recognized as an important contributor to innate immune responses against enteric pathogens. In combination with IL-10, IL-22 promotes barrier restitution. We have now linked EGCG administration with both and IL-10 and IL-22 production, changes that correlate with favorable alterations in TJP composition. Particularly, we have confirmed that elevated IL-22 production contributes to an upregulation of claudins-1and -5, while suppressing claudin-2. The evidence linking EGCG administration to enhanced IL-10 and -22 production provides additional insight into the clinical benefits seen from EGCG on IBD and pouchitis.

Effect of EGCG (5mcg/ml) on production of cytokines (ELISA) and their RNA message (RT-PCR)

Cytokine change from LPS stimulated baseline				
IL-22	IL-10	IL-12	IL-17A	IL-23
+28%	+55%	-45%	-18%	-37%
Message change from LPS stimulated baseline				
+24%	+22%	-18%	+4%	-25%

Measuring effects of EGCG 5mcg/ml added after stimulation with LPS 10ng/ml

Role of EGCG and LPS on TJP Production

	Occludin	Zonula occludin	Claudin-1	Claudin-2
Control	100	100	100	100
+EGCG	+64%	+32%	0%	-25%
+LPS	-64%	-55%	-65%	+200%
+LPS + EGCG	+28%	+20%	0%	-30%

Tu1784

THE SEVERITY OF MURINE *TOXOPLASMA GONDII*-INDUCED CROHN'S-LIKE ILEITIS IS MODULATED BY P2X7 SIGNALING

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Background and aims: Oral infection with *Toxoplasma gondii* cysts triggers a Crohn's disease (CD)-like enteritis in mice, associated with systemic inflammatory response and increased morbidity and mortality. Previous data from our group have demostrated an overexpression of ATP-activated P2X7 receptors in the colonic mucosa of patients with CD, whereas purinergic signaling was shown to be critically important in the development of experimental colitis. We hypothesized that the P2X7 receptor (P2X7-R) could also regulate key elements in CD-associated ileitis. **Methods:** Surgical samples obtained from patients with CD and controls were used to analyze histological and morphological parameters, and P2X7-R expression by real-time PCR and immunohistochemistry. For inducing experimental ileitis, wild type and P2X7-R knockout C57BL/6 mice were orally infected with 10 cysts of *T. gondii* ME49 strain each. After a follow-up of 8 days, animals were euthanized and ileal samples collected for several analyses, including histopathology, immunohistochemistry, nitric oxide (NO) and lactic dehydrogenase (LDH) levels, myeloperoxidase (MPO) activity, imflammatory mediators in the supernatants of explant cultures, and gene expression by qPCR. **Results**: Inflamed ileal mucosa of CD patients showed reduction in Paneth cells numbers and granules, goblet cells, but an increase in collagen fibers deposition, compared to controls. The overall P2X7-R expression was increased by qPCR, and distributed throughout the epithelium and the lamina propria mononuclear cells. Compared to uninfected controls, all *T. gondii*-infected animals lost more weight, and ileal samples had higher inflammatory scores, with less goblet cells, reduced Paneth cells numbers and granules, increased apoptotic rates and mononuclear cell infiltrates within the mucosa. NO, LDH, and MPO activity were significantly increased in ileal extracts from infected mice. A typical Th1/Th17 immune response was observed among the infected animals, based on measurements of inflammatory mediators from explant culture supernatants. However, P2X7-R knockout infected mice presented a greater parasitic burden within the ileum, in addition to a remarkably greater length of intestinal injury. **Conclusion:** Although P2X7-R overexpression may be implicated in the inflammatory response underlying human CD ileitis, the lack of P2X7-R signaling further increases the severity of intestinal lesions in the experimental model. In addition to amplifying inflammation, P2X7-R appears to play a critical role in the defense against *T. gondii* challenge, either directly or through changes in the gut microbiota.

Tu1785

THE ANTI-INFLAMMATORY EFFECT OF ADENOSINE A3 RECEPTOR BY MODULATING SEROTONIN AVAILABILITY IN COLONIC MUCOSA OF PATIENTS WITH ULCERATIVE COLITIS

Tianhua Ren, Minmin Lv, Xiaomeng An

OBJECTIVE: There is experimental evidence for the role of adenosine A3 receptor (A3AR) in colitis animal models. However, there is still a huge gap in our knowledge of human pathophysiology of A3AR signaling in ulcerative colitis (UC). Dysfunctional mucosal serotonin signaling has been implicated in pathogenesis of UC. This study aims to investigate the effect and the underlying mechanisms of A3AR on colonic mucosa of patients with UC. METHODS: Colonoscopy samples obtained from patients with UC and controls were treated with A3AR agonist 2-Cl-IB-MECA. A3AR expression was analyzed by immunofluorescence and Western blot. Inflammatory response was determined by the levels of pro-inflammatory cytokines TNF-α and IL-1β by real-time quantitative PCR in cultures of colonic mucosa explants. Colonic enterochromaffin (EC) cell density, tryptophan hydroxylase (TPH) expression,and serotonin content were investigated to identify the effect of 2-Cl-IB-MECA on colonic serotonin availability. RESULTS: The A3AR expression levels were significantly reduced in colonic epithelia of UC. 2-Cl-IB-MECA markedly depressed the expression levels of TNF-α and IL-1β in the colonic mocosa. In addition, the abnormal EC cell numbers, TPH expression and serotonin contents in colonic mucosa were significantly reduced by 2-Cl-IB-MECA. CONCLUSION: The findings demonstrate that the anti-inflammatory effect of A3AR agonist on colonic mucosa from patients with UC may be mediated via reducing EC cell hyperplasia and serotonin content. The important role of A3AR in regulating serotonin availability may provide an alternative therapy for UC.

Tu1786

INCREASED DEGRADATION OF GLUGAGON-LIKE PEPTIDE 1 IN RESPONSE TO COLONIC INFLAMMATION CONTRIBUTES TO IMPAIRED GLUCOSE METABOLISM IN MOUSE MODEL OF COLITIS

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Introduction: The role of incretin hormone glucagon-like peptide 1 (GLP-1) in inflammatory bowel diseases (IBD) development and exacerbations is still poorly understood. GLP-1, secreted by intestinal L cells, decreases blood glucose level and is degraded by dipeptidylpeptidase IV (DPP-IV). IBD patients may be at increased risk of impaired glucose metabolism due to the overproduction of pro-inflammatory cytokines and excessive administration of diabetogenic drugs. Moreover, GLP-1 may play a role as an anti-inflammatory agent. Thus, targeting incretin system may bring double benefits for therapy of IBD patients. **Aims:** The primary aim of this study was to investigate whether colitis impairs glucose metabolism in mice. The secondary goal was to assess the potential role of incretin hormones as an underlying factor in disturbances of glucose metabolism. **Methods:** Colitis was induced by intracolonic administration of trinitrobenzene sulfonic acid (TNBS) (first dose: 150 mg/kg at day 1, booster dose: 75 mg/kg at day 12). On day 15 mice were sacrificed and body weight, macroscopic score, ulcer score, colon length and bowel thickness were recorded. The expression of inflammatory molecules TNF-α, IL-1β, IL-6 and IL-17 was determined. The effect of colitis on glucose metabolism was studied by assessing fasting glucose levels,

mix group. (C) Principal coordinate analysis shows distinct microbial structure between the DSS group and butyrate or SCFA mix groups. (D, E) Relative abundance of bacteria at the phylum and species level based on metagenomic sequencing data.

Mo1099

AN *ESCHERICHIA COLI* STRAIN WITH EXTRA CATALASE ACTIVITY PROTECTS MICE FROM COLITIS THROUGH ROS-SCAVENGING

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Background / Aim: Inflammatory bowel disease (IBD), a chronic inflammatory disease of the gastrointestinal tract, is caused by various factors including genetic factors, environmental factors, immune responses, microbial changes, and oxidative stress. Recently, microbial targeted therapies have emerged as an alternative to immunosuppressive therapy for IBD. Here, we demonstrated the beneficial effects of an atypical *Escherichia coli* (*E.coli*) strain, which has an extra catalase gene compared to typical *E.coli* strain, against dextran sulfate sodium (DSS)-induced colitis in mice. **Method:** Mouse colitis was induced using DSS and confirmed by disease activity index and histopathology. Macrophages and T-cell subsets in isolated peritoneal cavity cells (PCCs) and splenocytes were analyzed by flow cytometry. Gene expression and cytokine profiles were determined using quantitative RT-PCR. Gene of 16S rRNA was subjected to MiSeq Illumina sequencing. **Results:** We found that the atypical *E.coli* administration in DSS colitis mice significantly restored body weight and colon length, reduced disease activity score, improved histological scoring score after periodic acid-schiff staining and decreased proinflammatory cytokine levels. The PO1 signal indicated noticeably decreased reactive oxygen species (ROS) in the DSS-treated atypical E.coli group compared to DSS-treated group. The regulatory T cell population was increased and the M1 macrophage population was decreased in PCCs after administration of atypical *E.coli*. Also, the microbial composition of the intestine was changed after the administration of atypical *E.coli*. The *Firmicutes* phylum was significantly recovered and the *Proteobacteria* phylum tended to reduce the richness, although it was statistically not significant. However, in *Il17* knockout (KO) mice, the body weight, disease activity score was not improved after the atypical *E.coli* administration. Moreover, the upregulation of regulatory T cells and microbial composition change were not presented in *Il17* KO mice. These results demonstrated that IL-17 is necessary for therapeutic effects of atypical *E.coli*. **Conclusion:** Atypical *E.coli* could ameliorate the inflammation of the colon by modulating reactive oxygen species and immune responses including regulatory T cell and M1 macrophage activations, and microbial composition. Atypical *E.coli*, which functions as an ROS scavenger, could be used as a therapeutic probiotics for IBD treatment. **Keywords:** reactive oxygen species, regulatory T cell, microbiome, probiotics, inflammatory bowel disease

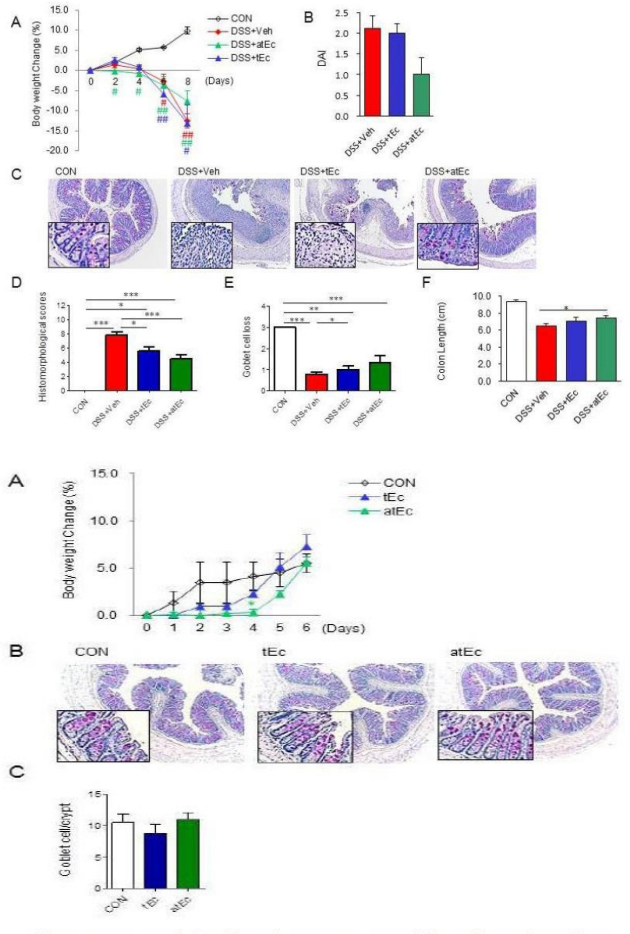

Figure 2. Atypical *E.coli* tends to increase goblet cells in the colon.

Mo1100

ESCHERICHIA COLI NISSLE 1917 EXPRESSING ELAFIN AMELIORATES THE INFLAMMATION IN COLONIC MUCOSA AND ENHANCES THE MUCOSAL EPITHELIAL BARRIER IN CHRONIC MICE COLITIS

Guigen Teng, Zilin Liu, Yun Liu, Weihong Wang

BACKGROUND: The increasing incidence of inflammatory bowel disease (IBD) underlines the need for new therapeutic methods. Probiotic *Escherichia coli* Nissle 1917 (EcN) as a way to treat IBD has its own limitations. Several studies have comfired the efficacy of Elafin in treating mice colitis models. However, no study has fully elucidated the mechanism of Elafin's beneficial effect against IBD. We used EcN to deliver Elafin in colonic mucosa and investigated the utility of Elafin-expressing EcN in ameliorating chronic mice colitis and the mechanism of Elafin in attenuating inflammation in colonic mucosal epithelial cells. **METHODS:** EcN was used to deliver Elafin directly to the colonic mucosa of mice, and wild-type EcN and DSS-induced chronic mouse colitis model was used as controls. In vitro, we transferred human Elafin gene into Caco-2 cells through lentivirus-mediated gene transfer system. Expression of mRNA was determined using RT-qPCR and level of protein expression was either determined by immunohistochemistry or western blot. **RESULTS:** Elafin-expressing EcN treated mice displayed longer colon length than the DSS control group and wild-type EcN group was in between, but the results were not statistically significant. Elafin-expressing EcN reduced mRNA and protein expression of pro-inflammatory cytokines IL-8, TNF-α and IL-1β in colonic mucosa. Overexpressing Elafin in Caco-2 cells enhanced the mRNA expression of tight junction proteins ZO-1, Claudin-1 and Occludin. HNE-induced increases in the expression of EGFR, PI3K, AKT and IL-8 mRNAs in Caco-2 cells were reduced by coculturing with Elafin-expressing EcN. Moreover, the enhanced protein expression of NF-kB signaling pathway proteins (p-P65, Ikkβ/p-Ikkβ, IκBα/p-IκBα) through co-incubation with HNE was reduced by coculturing with Elafin-expressing EcN. **CONCLUSION:** Elafin-expressing EcN protects mice against chronic colitis and enhances the intestinal epithelial barrier through inhibition of NF-κB pathway via EGFR-AKT-PI3K signaling.

Mo1101

PROPHYLACTIC ADMINISTRATION OF PHENOLIC COMPOUNDS EXTRACTED FROM POWDER BEER ATTENUATES EXPERIMENTAL COLITIS

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Background and aims: Accumulated evidence indicate that diet plays an important role in the pathogenesis of inflammatory bowel disease (IBD), as a critical risk factor, but it also constitutes a potential opportunity for therapeutic interventions. Phenolic compounds present in food, such as teas, beer, wine, olive oil, chocolate/cocoa, coffee, nuts, pomegranate, corn, among others, have been associated with a decreased risk for the development of cancer, type 2 diabetes, and inflammatory, allergic, cardiovascular, and neurodegenerative diseases. The aim of this study was to investigate the effect of pre-treatment with oral phenolic compounds extract from the powder of craft beer in experimental colitis induced in mice by dextran sodium sulfate (DSS). **Methods:** We first extracted and quantified phenolic compounds from pure beer samples, which were characterized as galic acid, di-OH-benzoic, 4-OH-fenilacetic, vanilic acid, M-cumaric, P-cumaric, 5-CQA-clorogenic, ferulic acid, siringic acid and rosmarinic acid. Then, Balb/c mice received either the beer phenolic extract (BPE) of phenolic compounds or the beer powder fortified with phenolic extract (BPFPE) of phenolic compounds daily for twenty days, by gavage. On day 21, mice were induced to experimental colitis by receiving 2.5% dextran sodium sulfate (DSS) in drinking water for 7 days. Two additional groups of animals not submitted to DSS, or not receiving BPE or BPFPE served as normal and inflammatory control groups, respectively. Body weight and food ingestion were assessed daily during colitis induction. On day 28, animals were euthanized and distal colon samples were collected for histopathological analysis; staining for goblet cells; and immunohistochemistry characterizing mononuclear cells, apoptotic cells, proliferating cells and intracellular pathway activation. **Results:** Mice induced with DSS lost more weight, reduced the food ingestion, and had reduced colon length, with dense inflammatory cell infiltrates, ulcerations, increased crypt damage, and loss of goblet cells, compared to BPE and BPFPE pre-treated animals. In addition, in DSS-induced mice, densities of CD4- and CD11b-positive cells, apoptotic rates, and activation of NF-kappa B and Erk1/2 MAPK intracellular signaling pathways were significantly increased, compared to BPE and BPFPE pre-treated animals. **Conclusion:** Pre-treatment with BPE and BPFPE remarkably attenuates DSS-induced colitis in mice, by preserving mucosal integrity and with less inflammatory cell infiltration based on the activation of NF-kappa B and MAPK signaling pathways. The protective effect of phenolic compounds extracted from powder beer support the idea of further investigating and developing therapies for human IBD based on dietary interventions.

Mo1103

EFFECT OF WEICHANG'AN PILL ON TH17/TREG IMMUNE BALANCE IN MICE WITH ULCERATIVE COLITIS

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Background: Weichang'an Pill is a traditional Chinese medicine preparation widely used in digestive system diseases. Our previous experimental studies have found that Weichang'an Pill can effectively attenuate intestinal symptoms in rats with ulcerative colitis, such as diarrhea, rectal bleeding, etc. It can repair intestinal mucosal injury and promote ulcer healing, but whether its mechanism is related to immunity is not clear. **Objective:**To explore the mechanism of Weichang'an Pill in the treatment of ulcerative colitis from the perspective of immune balance between regulatory T cells and helper T17 cells. **Methods:** Sixty C57BL/6 mice, except 10 normal mice, were free to drink 2.5% sodium DSS for 13 days to replicate ulcerative colitis model. After the model evaluation, they were randomly divided into model, mesalazine, high dose, medium dose and low dose group. Then the mesalazine group was

27

THE P2X7 RECEPTOR MODULATES INFLAMMATION AND COLLAGEN DEPOSITION IN EXPERIMENTAL CHRONIC COLITIS AND COLOCALIZES WITH HUMAN IBD COLONIC MYOFIBROBLASTS

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Background: A usual outcome in the chronic inflammatory microenvironment of Inflammatory Bowel Diseases (IBDs) is intestinal fibrosis, which can lead to complications such as stenosis and obstruction. Extracellular ATP can act as a damage-associated molecular pattern, and signal cellular stress through the binding with purinergic receptors, such as P2X7-R. Previous works show that the P2X7-R is overexpressed in the mucosa of patients with IBD, particularly in Crohn's Disease (CD), and that its blockade in experimental models prevents the development of intestinal inflammation. Aims: This work aims to analyse the involvement of the ATP-P2X7 receptor pathway in the development of chronic inflammation and intestinal fibrosis. Methods: Wild C57BL/6 ($P2X7^{+/+}$) and P2X7-R genetic deficient ($P2X7^{-/-}$) mice were subjected to chronic colitis by the cyclic administration of 2% Dextran Sodium Sulfate (DSS) in drinking water. Three cycles of a week-long exposure to DSS, followed by two weeks of pause were performed. After induction, mice were submitted to colonoscopy and endoscopic ultrasound to assess disease activity and wall stiffness. Analyses included histological score and goblet cell and collagen staining. Cytokine measurement on colon specimen supernatants was performed using a CBA Th1/Th2/Th17 kit. Colonic biopsies from IBD patients and controls were obtained for double immunofluorescence under confocal microscopy for ubiquitous mesenchymal cell markers. Results: DSS-treated mice presented macroscopic changes compatible with inflammation activity, with a higher colonoscopic score in $P2X7^{+/+}$ mice compared to $P2X7^{-/-}$ mice ($p< 0.01$). Upon ultrasound examination, after intrarectal injection of 0.5 mL of saline, a lower variation on the cross-sectional area of the colon of $P2X7^{+/+}$ mice was observed ($p = 0.005$). Histological score and collagen density were found to be higher in wild-type group ($p< 0.01$), as goblet cell density was lower in this group ($p< 0.01$). Activation of NFkB, NLRP3 and Caspase-1 signalling pathways were lower in colon tissues from $P2X7^{-/-}$ mice, as well as collagen concentration measured in colonic supernatant ($p< 0.05$). Inflammatory cytokines such as IL-1beta and IFN-gamma were lower in $P2X7^{-/-}$ mice ($p< 0.001$), as well as anti-inflammatory cytokines such as IL-10 ($p< 0.001$). No significant difference was found for TNF-alpha, IL-6 and IL-17. Co-localization studies under confocal analysis of colon tissues from IBD-patients showed a higher density of $P2X7^{+}$/alpha-SMA^{+} cells compared to controls, more so in CD ($p< 0.01$). Conclusion: The P2X7-R is overexpressed in the chronic inflamed colon of human IBD, and co-localizes predominantly with myofibroblasts. The experimental model appears to corroborate the role of P2X7-R in amplification of the inflammatory process with the induction of a fibrotic response in mice subjected to chronic colitis.

28

LOSS OF PTPN2 COMPROMISES THE ABILITY OF MACROPHAGES TI CLEAR ADHERENT-INVASIVE *E. COLI*

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Background/aims: A wide range of autoimmune and inflammatory conditions, including inflammatory bowel diseases (IBD), are associated with alterations in the intestinal microbiota, and especially IBD is characterized by increased abundance and mucosal penetration of pathobionts, such as invasive-adherent *E. coli* (AIEC) species. At mucosal surfaces, macrophages are key players that remove invading pathogens and maintain immune homeostasis. In IBD patients, however, intestinal macrophages show a compromised ability to clear invading pathogens resulting in elevated bacterial translocation and excessive and prolonged immune activation triggering chronic inflammatory processes. The aim of our study was to investigate how a loss-of-function variant in the protein tyrosine phosphatase non-receptor type 2 (*PTPN2*) gene, which is associated with an increased risk for IBD, or complete PTPN2-loss, affects the ability of macrophages to defeat invading bacteria. **Methods:** IBD patient-derived macrophages carrying wild-type *PTPN2* or the IBD-associated *PTPN2* rs1893217 SNP and peritoneal macrophages from WT and constitutive PTPN2-knockout mice, as well as mice specifically lacking *PTPN2* in macrophages (*Ptpn2*LysMCre), were infected with non-invasive K12 *E.coli*, the human AIEC *LF82*, or a novel mouse AIEC (*m*AIEC) strain. **Results:** Presence of the PTPN2 risk variant in patient-derived macrophages and PTPN2 knockout in murine peritoneal macrophages promoted pathobiont invasion/uptake ($p<0.001$, each) and intracellular survival/proliferation ($p<0.001$, each). PTPN2-deficient cells showed enhanced expression of carcinoembryonic antigen cellular adhesion molecule (CEACAM)1 and CEACAM6, and inhibition of these CEACAMs with blocking antibodies normalized bacterial uptake. Increased CEACAM expression was STAT1-dependent and STAT1-silencing significantly reduced bacterial invasion ($p<0.01$). PTPN2-deficient cells showed defective autophagy induction upon infection with AIEC ($p<0.05$) and severely compromised lysosomal acidification. Induction of autophagy using rapamycin, combined with promotion of lysosomal acidification restored the ability of PTPN2-deficient cells to clear intracellular bacteria. *In vivo* infection with *m*AIEC resulted in increased numbers of colony-forming units in mesenteric lymph nodes, liver and spleen ($p<0.01$, each), and increased bacterial uptake into lamina propria macrophages ($p<0.05$) in *Ptpn2*LysMCre mice and these mice were more susceptible to *m*AIEC-induced disease ($p<0.05$). **Conclusions:** Our findings reveal a tripartite regulatory mechanism involving CEACAM expression, autophagy, and lysosomal acidification, by which PTPN2 preserves macrophage anti-bacterial function. This indicates a central role for PTPN2 during host defense against invading bacteria.

29

GPR120 REGULATES $CD4^{+}$ T CELL IL-10 PRODUCTION TO CONTROL INTESTINAL INFLAMMATION

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Background: $CD4^{+}$ T cell production of IL-10 plays a critical role in maintaining intestinal homeostasis. G protein-coupled receptor (GPR) 120, a receptor for omega 3 fatty acids, has been implicated in regulating metabolic syndromes with anti-inflammatory function. However, the role of GPR120 in intestinal inflammation is unknown. In this study, we investigated whether and how GPR120 regulates $CD4^{+}$ T cell function to maintain intestinal homeostasis. **Methods**: Dextran sodium sulfate (DSS) induced colitis model and *Citrobacter rodentium* infection model were used to compare intestinal inflammation between wild-type (WT) and *Gpr120*$^{-/-}$ mice, or WT *Cd4*^{cre} *Gpr120*$^{fl/+}$ and $CD4^{+}$ T cell-specific GPR120 deficient *Cd4*^{cre} *Gpr120*$^{fl/fl}$ mice. $CD4^{+}CD45RB^{hi}$ T cell adoptive transfer model was utilized to analyze the pathogenesis of WT and GPR120-deficient $CD4^{+}$ T cells in inducing colitis. $CD4^{+}$ T cells were treated with or without GPR120 agonist, CpdA, and the gene expressions were analyzed by RNA sequencing and qRT-PCR. Seahorse was used for the measurement of T cell metabolism. Blimp1-deficient $CD4^{+}$ T cells isolated from *Cd4*^{cre} *Prdm1*$^{fl/fl}$ mice were used for mechanistic studies. Mice were administered GPR120 agonist for investigating the potential treatment of GPR120 agonist in treating intestinal inflammation. **Results**: We demonstrated that deficiency of GPR120 resulted in more severe colitis in mice upon DSS insults and *Citrobacter rodentium* infection. Compared with other cell types, $CD4^{+}$ T cells expressed GPR120 at a high level. Similar to whole body GPR 120 knock-out mice, $CD4^{+}$ T cell-specific GPR120 deficient *Cd4*^{cre} *Gpr120*$^{fl/fl}$ mice were more susceptible to colitis development than WT *Cd4*^{cre} *Gpr120*$^{fl/+}$ mice. Transfer of GPR120-deficient $CD4^{+}CD45Rb^{hi}$ T cells induced more severe colitis in *Rag*$^{-/-}$ mice with increased IL-17 and IFNg producing $CD4^{+}$ T cells and decreased IL-10 producing $CD4^{+}$ T cells in intestinal lamina propria than the transfer of WT T cells. Treatment with GPR120 agonist, CpdA, promoted $CD4^{+}$ T cell production of IL-10 by upregulating Blimp1 and enhancing glycolysis, which were regulated by mTOR. Consistently, docosahexaenoic acid (DHA), a dietary omega 3 fatty acid, also upregulated the IL-10 production in $CD4^{+}$ T cells. GPR120 agonist-treated WT but not Blimp1-deficient Th1 cells induced less severe colitis. Importantly, oral administration of CpdA protected mice against intestinal inflammation, indicating that Blimp1 mediates GPR120 promotion of $CD4^{+}$ T cell IL-10 production and inhibition of colitis development. **Conclusions:** Our findings demonstrate the role of GPR120 in regulating intestinal $CD4^{+}$ T cell function to maintain intestinal homeostasis through induction of IL-10 production, which identifies GPR120 as a potential therapeutic target for IBD treatment.

30

A NOVEL MECHANISM OF SPHINGOSINE-1-PHOSPHATE RECEPTOR (S1PR) MODULATORS IN INFLAMMATORY BOWEL DISEASES

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Background: S1P receptor (S1PR) modulators are a novel form of treatment for inflammatory bowel disease (IBD) patients, and have been successfully tested in phase 2 clinical trials in ulcerative colitis (UC). Modulation of $S1P_1$ is believed to lead to lymphocyte redistribution in peripheral lymphoid tissues and reduction in the number of circulating lymphocytes. However, a lack of correlation between reduction of circulating lymphocytes and clinical efficacy suggests additional mechanisms of action. We hypothesized S1PR modulators may act directly at the intestinal mucosa level. **Methods**: Intestinal resection specimens from IBD patients and non-IBD controls were collected and expression levels of $S1P_{1-5}$ analyzed via qPCR and staining. Primary human intestinal myofibroblasts (HIMF), muscle cells (HIMC), lamina propria mononuclear cells (LPMC) and microvascular endothelial cells (HIMEC) were isolated and their expression of S1PRs examined by qPCR. Effects of S1P and S1PR modulators on HIMEC function was evaluated by migration and proliferation assays (scratch assay, ^{3}H-thymidine), cytokine production (IL-8 ELISA) and signal transduction (immunoblot). Effects of the same modulators on cytokine production (ELISA) were also examined in a human intestinal biopsy culture system. **Results:** $S1P_1$ exhibited higher gene expression in UC and Crohn's disease (CD) intestinal tissues compared to non-IBD controls ($p<0.001$), which was confirmed at the protein level by IHC. $S1P_1$ was detected in both intestinal immune and non-immune cells, with HIMEC expressing the highest levels, moderate levels in LPMC and lower expression in HIMF and HIMC. S1P promoted migration (1.5-fold over control, $p<0.05$) and proliferation (1.6-fold, $p<0.05$) of HIMEC, indicative of a pro-angiogenic effect. Treatment of HIMEC with an $S1P_{1/5}$ modulator inhibited cell migration and proliferation by 75% and 70%, respectively. In addition, this$S1P_{1/5}$ modulator decreased IL-8 secretion ($p<0.01$), ICAM-1 and VCAM-1 expression ($p<0.05$) and attenuated the Erk signaling pathway. None of these effects were observed when HIMEC were treated with a selective $S1P_5$ modulator, suggesting that these inhibitory effects were mediated by the S1P/$S1P_1$ pathway. Inhibition of cytokine secretion was also observed in the intestinal biopsy system, where S1P-induced IL-8 production decreased when the $S1P_{1/5}$ modulator was added to the culture. **Conclusions**: The results point to a potential novel mechanism of action of S1PR modulators in IBD through the inhibition of angiogenesis and IL-8 secretion in IBD tissues induced by $S1P_1$ modulation. This selective activity, combined with the reduction numbers of circulating lymphocytes, may contribute to the clinical efficacy of S1PR modulation in IBD patients.