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DISSERTAÇÃO DE MESTRADO EM BIOLOGIA MOLECULAR

AVALIAÇÃO *IN VIVO* DO POTENCIAL IMUNOMODULADOR DE β-GLUCANA DE *Auricularia auricula* EM MODELO MURINO DE INFECÇÃO TIPO SEPSE

ALUNO: JESSE PEREIRA MACHADO VIANA
ORIENTAÇÃO: DRA. ANAMÉLIA LORENZETTI BOCCA
CO-ORIENTAÇÃO: DRA. MÁRCIA CRISTINA GONÇALVES MACIEL

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Dissertação de mestrado apresentada como requisito parcial para obtenção do título de Mestre em Ciências Biológicas (Biologia Molecular) pela Universidade de Brasília (UnB).

Orientação: Dra. Anamélia Lorenzetti Bocca

Co-orientação: Dra. Márcia Cristina Gonçalves Maciel

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RESUMO

A sepse é definida como disfunção orgânica com risco de vida causada por uma resposta desregulada do hospedeiro à infecção. Essa condição é potencialmente fatal e o tratamento desafiador, necessitando de abordagens alternativas que possam reduzir a carga do agente infeccioso e modular a resposta imunológica do paciente. Nesse contexto, as β-glucanas surgem como candidatos potenciais, devido às suas atividades biológicas, em especial a atividade imunomoduladora e antimicrobiana. **Objetivo:** Investigar o efeito do tratamento com β-glucana de *Auricularia auricula* em modelo de infecção sistêmica do tipo sepse letal. **Métodos**: Capítulo I - Foi realizada uma revisão integrativa da literatura baseada em etapas sistemáticas, com buscas realizadas nas bases de dados PubMed, ScienceDirect, Scopus, Web of Science e Embase. As buscas foram conduzidas usando palavras-chave e descritores controlados pelo Medical Subject Headings (MeSH) e os termos não controlados "glucan," "sepsis," e "cecal ligation and puncture," juntamente com os operadores booleanos "AND" e "OR," adaptando-os a cada base de dados específica quando necessário. A plataforma Rayyan foi utilizada para organizar os artigos obtidos e auxiliar na exclusão de artigos fora dos critérios de seleção. Capítulo II (Será submetido Microbial Pathogenesis) - Uma preparação contendo β-glucana, oriunda do basidiomiceto *Auricularia auricula*, foi administrada via gavagem, por 15 dias antes da indução da sepse, na dose de 5 mg/kg como forma de tratamento profilático. Os camundongos (C57BL/6) fêmeas foram divididos em 3 grupos: SHAM (falso-operado), CLP (sepse induzida pelo modelo de ligadura e perfuração cecal - sem tratamento) e CLP GLU (sepse induzida pelo modelo CLP, e tratamento administrado via gavagem 15 dias antes da indução da sepse). **Resultados**: Capítulo I - Na maioria dos estudos, o principal tipo de glucana investigada foi a β-glucana (88,2%), foi descrito a redução de citocinas inflamatórias (TNF-α e IL-6), aumento de atividade antioxidante, redução da ocorrência de danos teciduais, levando ao aumento da sobrevivência animal. Capítulo II - O tratamento com β-glucana estimula a proliferação dos linfócitos e granulócitos circulantes no sangue, atenua os danos pulmonares causados pela sepse, reduz as CFU's no peritônio e sangue, evita a ocorrência de plaquetopenia, regula os níveis séricos de citocinas e prolonga a sobrevida de camundongos sépticos. **Conclusões:** Em conjunto, os dados apresentados nos capítulos I e II indicam que a β-glucana é um nutracêutico que representa uma nova estratégia terapêutica com potencial para modular a resposta imunológica, controlar a proliferação microbiana e melhorar a sobrevida em um estado de sepse grave, induzida em camundongos. β-glucana pode ser considerado uma alternativa terapêutica para estimular e/ou modular a resposta imune em quadros de sepse, pode também atuar como adjuvante ao tratamento do uso de fármacos padrão na clínica, contribuindo assim com um melhor prognóstico em sepse letal.

Palavras-chave: *Auricularia auricula*; beta-glucana; sepse; infecção; imunomodulação;

ABSTRACT

Sepsis is defined as life-threatening organ dysfunction caused by a deregulated host response to infection. This condition is potentially fatal and treatment challenging, requiring alternative approaches that can reduce the burden of the infectious agent and modulate the patient's immune response. In this context, β-glucans have emerged as potential candidates due to their biological activities, especially their immunomodulatory and antimicrobial activity. **Objective**: To investigate the effect of treatment with β-glucan from *Auricularia auricula* in a lethal sepsis-type systemic infection model. **Methods**: Chapter I - An integrative literature review based on systematic steps was carried out, with searches conducted in the PubMed, ScienceDirect, Scopus, Web of Science and Embase databases. The searches were conducted using keywords and descriptors controlled by the Medical Subject Headings (MeSH) and the uncontrolled terms "glucan," "sepsis," and "cecal ligation and puncture," along with the Boolean operators "AND" and "OR," adapting them to each specific database when necessary. The Rayyan platform was used to organize the articles obtained and to help exclude articles that did not meet the selection criteria. Chapter II (Microbial Pathogenesis will be submitted) - A preparation containing β-glucan from the basidiomycete *Auricularia auricula* was administered via gavage for 15 days before inducing sepsis, at a dose of 5 mg/kg as a form of prophylactic treatment. The female mice (C57BL/6) were divided into 3 groups: SHAM ("false-operated"), CLP (sepsis induced by the cecal ligation and perforation model - no treatment) and CLP GLU (sepsis induced by the CLP model, and treatment administered by gavage 15 days before the induction of sepsis). **Results**: Chapter I - In the majority of studies, the main type of glucan investigated was β-glucan (88.2%), a reduction in inflammatory cytokines (TNF-α and IL-6), an increase in antioxidant activity, a reduction in the occurrence of tissue damage, leading to an increase in animal survival were described. Chapter II - Treatment with β-glucan stimulates the proliferation of circulating lymphocytes and granulocytes in the blood, attenuates lung damage caused by sepsis, reduces CFU in the peritoneum and blood, prevents the occurrence of thrombocytopenia, regulates serum cytokine levels and prolongs the survival of septic mice. **Conclusions**: Taken together, the data presented in Chapters I and II indicate that β-glucan is a nutraceutical that represents a new therapeutic strategy with the potential to modulate the immune response, control microbial proliferation and improve survival in a state of severe sepsis induced in mice. β-glucan can be considered a therapeutic alternative to stimulate and/or modulate the immune response in cases of sepsis, and can also act as an adjuvant treatment to the use of standard drugs in the clinic, thus contributing to a better prognosis in lethal sepsis.

Keywords: *Auricularia auricula*; beta-glucan; sepsis; infection; immunomodulation;

LISTA DE ABREVIATURAS E SIGLAS

UTI: Unidade de Terapia Intensiva;

ANVISA: Agência Nacional de Vigilância Sanitária;

SIRS: Síndrome da Resposta Inflamatória Sistêmica;

ACCP: American College of Chest Physicians;

SCCM: Society of Critical Care Medicine;

$PaCO_2$: Pressão Parcial De Dióxido de Carbono;

ESICM: European Society of Intensive Care Medicine;

SOFA: Sequential Organ Failure Assessment;

qSOFA: Quick SOFA;

ILAS: Instituto Latino Americano de Sepse;

MODS: Síndrome da Disfunção de Múltiplos Órgãos;

PRR: receptor de reconhecimento de padrão;

DAMP, damage-associated molecular pattern;

PAMP, pathogen-associated molecular pattern;

PD-1: programmed cell death protein 1;

PD-L1: programmed death ligand 1;

CTLA-4: cytotoxic T lymphocyte antigen-4;

EUA: Estados Unidos da América

CR3: receptor de complemento 3;

TLR: toll like receptor;

NK: natural killers;

EPS: exopolissacarídeo;

DPPH: 2,2-difenil-1-picrilhidrazil

BMDMs: macrófagos derivados da medula óssea;

CLRs: receptores de lectinas tipo-c

Syk: tirosina cinase do baço

CARD9: complexo proteico da família de recrutamento de caspase 9;

BCL10: linfoma/leucemia de células B 10;

MALT1: proteína 1 de translocação de linfoma de tecido linfóide associado à mucosa;

PI3K: fosfatidilinositol-3-cinase;

AKT/PKB: proteína quinase B;

NF-κB: fator nuclear kappa B;

PLC-γ2: fosfolipase C gama 2;

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1. INTRODUÇÃO

A sepse é uma disfunção orgânica gravíssima que apresenta risco de vida ao hospedeiro, ela surge em decorrência de uma resposta imunológica desregulada frente a uma infecção. O choque séptico é sua forma agravada, que ocorre quando anormalidades circulatórias, celulares e metabólicas induzidas pela infecção causam disfunção orgânica grave e risco substancial de morte (Singer et al., 2016).

Os dados epidemiológicos da sepse variam de acordo com a região e a população estudada. Em 2020, a pesquisa Global Burden of Sepsis calculou 48,9 milhões de casos de sepse ocorridos globalmente, em 2017 (Rudd et al., 2020). Metade dos casos ocorreu em crianças, sendo a maioria delas menores de cinco anos. Ainda nesse ano, estimou-se que 19,8% das mortes globais eram sepse, representando 11,0 milhões de óbitos. Conforme a pesquisa Global Burden of Sepsis, a incidência de sepse e a taxa de mortalidade relacionada apresentaram uma redução de 37,0 % e 52,8%, respectivamente, entre 1990 e 2017. No Brasil, uma análise epidemiológica constatou 113.059 mil óbitos registrados por sepse, entre 2018 e 2022. Com maior prevalência entre mulheres (51,16%), indivíduos de cor branca (52%), idosos com idade ≥80 anos (37,9%), e residentes na região Sudeste (52,96%). Também foram relatadas 660.257 hospitalizações, com maior prevalência no sexo masculino (52,16%), entre esses os de cor branca (36,54%), com idade ≥80 anos (21,07%) e residentes nas regiões Sudeste (51,08%), onde o caráter de atendimento foi a urgência (95,58%). (dos Santos et al., 2024). Dado o exposto, observa-se como visão geral de dados disponíveis publicamente, que a sepse continua a ser um grande problema de saúde pública em todo o mundo, com altas taxas de incidência e mortalidade.

O tratamento convencional dado aos pacientes sépticos inclui o uso de antimicrobianos de amplo espectro, ressuscitação volêmica e suporte hemodinâmico (Rhodes et al., 2017). Entretanto, ainda existem dificuldades no tratamento que eventualmente levam à morte dos pacientes: como a resistência aos antibióticos (Legese et al., 2022) e a disfunção imunológica induzida pela sepse (Mithal et al., 2022). Além disso, há fatores de risco associados a piora do quadro séptico, como a idade avançada e presença de comorbidades, por exemplo, doenças cardíacas e pulmonares (Rowe & McKoy, 2017), bem como a imunossupressão induzida (Torres et al., 2022) que incorrem na piora do quadro séptico. Tais fatores, além de

contribuirem para o agravamento do quadro séptico, impõem um ônus significativo aos sistemas de saúde, devido aos altos custos relacionados ao tratamento e internações em unidades de terapia intensiva (UTI) (Angus et al., 2001).

Nos últimos anos, pesquisadores têm estudado exaustivamente estratégias eficazes para tratar pacientes com sepse e restaurar o equilíbrio imunológico. Uma estratégia promissora é o uso de produtos naturais com potencial anti-inflamatório e/ou imunomodulador. Nesse contexto, surgem as β-glucanas (Lull et al., 2005), elas são consideradas prebióticos. A Agência Nacional de Vigilância Sanitária (ANVISA) em 1999 regulamentou o Alimento Funcional "[...] como um alimento ou ingrediente que, além das funções nutricionais básicas, quando consumido como parte da dieta usual, produz efeitos metabólicos e/ou efeitos benéficos à saúde, devendo ser seguro para consumo sem supervisão médica" (Sanitária, 2018). As β-glucanas já são amplamente comercializadas no Brasil, muitas vezes em forma de suplementos alimentares destinados a fortalecer o sistema imunológico. Um exemplo é a β-glucana da Biolab, oferecida em cápsulas com 765 mg de β-glucana. Ela é extraída da levedura *Saccharomyces cerevisiae* e purificada com vitaminas A, E, D3 e Zinco. Outro produto disponível é a β-glucana da BioVittare, oferecida em cápsulas de 250 mg, indicada para o reforço do sistema imunológico e melhora de alergias. Esses suplementos são facilmente encontrados em farmácias e lojas especializadas, reforçando suas facilidades no mercado como coadjuvantes no fortalecimento imunológico.

Estudos mostram que o tratamento com β-glucanas apresenta efeito protetor, regulando células supressoras mieloides (MDSCs) (Liu et al., 2017) e modulando citocinas importantes para o quadro inflamatório, como IL-6, IL-10 e TNF-α (Senoglu et al., 2008). Um estudo investigou os efeitos de uma suplementação oral de β-glucanas em camundongos infectados via intraperitoneal com *Escherichia coli* (Walachowski et al., 2022). Os dados apresentados indicaram melhora no combate à infecção, os grupos tratados apresentaram menor carga bacteriana e diminuição do dano tecidual associado à resposta inflamatória exacerbada. Outros estudos também demonstraram efeitos protetores associados ao tratamento com β-glucanas contra danos pulmonares associados à sepse, reduzindo as porcentagens de neutrófilos e linfócitos do lavado broncoalveolar, além de atenuar a liberação de citocinas

inflamatórias, prevenir lesão pulmonar aguda e aumentar a taxa de sobrevivência de animais sépticos (Babayigit et al., 2005).

A β-glucana utilizada nesse trabalho tem origem do cogumelo *Auricularia Auricula,* um basidiomiceto que apresenta atividade anticancerígena (Kang et al., 2020; Liu et al., 2021), antidiabética (Xu et al., 2021), imunoestimulatória (Kong et al., 2020), entre outras. Conforme caracterizado por (Basso, 2017) a composição monossacarídica da fração solúvel e insolúvel desse cogumelo é constituída por fucose, xilose, manose, galactose e glicose, com arabinose, presente apenas na fração insolúvel, e ribose, na fração solúvel. Além disso, na análise estrutural feita ressonância magnética nuclear foram identificados os açucares manose e glicose (β-D-Glcp 6 e α-D-Manp 2,6), com identificação do polissacarídeo beta-1,6-glucana. Portanto, a β-glucana utilizada nesse estudo é purificada, estabelecendo um padrão de qualidade em relação às β-glucanas comerciais de origem fúngica, que por sua vez podem conter resíduos de quitina em sua composição.

O grupo de pesquisa do Laboratório de Imunologia Aplicada (LIA-UnB) também já possui trabalhos publicados com o estudo de β-glucanas. Carbonero e colaboradores (Carbonero et al., 2024) descreveram a estrutura química e efeitos biológicos de glucanas presentes no Bionutri-AR1, como o α-D-Glucano 1→4 e β-D-Glucano 1→3, 1→6. Os resultados indicaram que ambos os glucanos apresentaram potencial antioxidante, atuando na eliminação de radicais livres 2,2-difenil-1-picrilhidrazil (DPPH). Além disso, foi identificada a capacidade de estimular a secreção das citocinas TNF-α, IL-6 e IL-10 em macrófagos derivados da medula óssea (BMDMs), exercendo sua atuação em vias inflamatórias. Da mesma maneira, outro estudo avaliou a atividade imunoestimulatória de exopolissacarídeos (EPS) contendo β-(1,3)-glucano de cogumelos comestíveis semelhantes ao *Auricularia auricula* em BMDMs de camundongos infectados com *Cryptococcus neoformans*, um fungo responsável por micoses graves em imunocomprometidos (Basso et al., 2020). Os resultados indicam que os EPS contendo β-(1,3)-glucanos exercem forte atividade imunomoduladora: ativando macrófagos e células dendríticas via receptor Dectina-1. Além disso, o tratamento induz repolarização de macrófagos para um perfil pró-inflamatório, aumentando a produção de óxido nítrico e a capacidade microbicida contra *C. neoformans*. Adicionalmente, observou-se maior transcrição

de genes associados à proteção do hospedeiro e melhoria significativa da sobrevivência de camundongos infectados após administração oral de EPS. Esses achados destacam a experiência do grupo de pesquisa no estudo com β-glucanas, além de reforçar em especial o potencial de β-glucanas como objeto de estudo no combate a infecções.

O estudo com produtos naturais, em especial β-glucanas, tem se mostrado cada vez mais importante, sugerindo uma estratégia promissora no combate a infecções, especialmente pelos papéis desempenhados na defesa, atividade anti-inflamatória e na modulação clínica da resposta imune do hospedeiro, possibilitando novos usos e o desenvolvimento de novas terapias de intervenção na sepse. Portanto, o presente estudo buscou aprofundar o conhecimento sobre os efeitos das β-glucanas no contexto da sepse, avaliando sua capacidade de modular a resposta imune de maneira a reduzir a inflamação exacerbada e melhorar os avanços clínicos. A melhor compreensão desses mecanismos poderá contribuir para o avanço de estratégias terapêuticas mais eficazes e menos dependentes de intervenções convencionais.

2. FUNDAMENTAÇÃO TEÓRICA

2.1. SEPSE

2.1.1. O conceito de sepse

Inicialmente, a definição de sepse estava fortemente associada à síndrome da resposta inflamatória sistêmica (SIRS). A primeira definição foi descrita em 1992 por especialistas do American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) e baseava-se na suspeita de infecção, com a presença de no mínimo 2 critérios da SIRS. Os critérios estabelecidos para SIRS eram: febre (temperatura corporal > 38°C) ou hipotermia (temperatura corporal < 36°C), taquipneia (frequência respiratória > 20 incursões respiratórias por minuto) ou pressão parcial de dióxido de carbono ($PaCO_2$) < 32 mmHg (4,3 kPa), ou taquicardia (frequência cardíaca > 90 batimentos por minuto), neutrofilia (contagem de leucócitos > 12.000/mm³) ou neutropenia (contagem de leucócitos < 4.000/mm³), ou presença de mais de 10% dos neutrófilos circulantes no sangue periféricos imaturos. Além disso, as formas mais graves, como a sepse grave e o choque séptico, foram definidas como: sepse grave é a "sepse associada à disfunção orgânica, anormalidade de hipoperfusão (acidose láctica, oligúria e alteração aguda do estado mental) ou

hipotensão induzida por sepse". E choque séptico é a "hipotensão induzida por sepse, persistindo apesar da ressuscitação fluídica adequada, juntamente com a presença de anormalidades de hipoperfusão ou disfunção orgânica" (Bone et al., 1992).

Entretanto, apesar de todos os esforços para definir e diagnosticar corretamente a sepse, a primeira definição destacava a ocorrência inflamatória, mas não entendia a complexidade do choque séptico e da disfunção orgânica. Sendo assim, ela não foi suficiente para distinguir os pacientes sépticos dos que apresentavam resposta inflamatória normal, ou de origem não infecciosa. Dada a necessidade de melhor entender a fisiopatologia da sepse, em 2003, a definição foi ampliada para incorporar critérios extras e um foco mais acentuado na disfunção orgânica. Por definição, sepse ainda continuava como uma resposta inflamatória sistêmica à infecção, mas indicadores clínicos e laboratoriais foram introduzidos com o intuito de simplificar o diagnóstico de sepse (Levy et al., 2003). Contudo, a definição ainda estava presa ao conceito de SIRS, que nem sempre está presente em pacientes sépticos e pode surgir em situações não infecciosas, como traumas, pancreatite ou queimaduras.

Por fim, a terceira e mais recente definição de sepse foi uma reformulação, que redefiniu completamente o conceito de sepse, abandonando o uso de SIRS como critério principal. Em 2016, a Society of Critical Care Medicine (SCCM) e European Society of Intensive Care Medicine (ESICM) conduziram O Terceiro Consenso Internacional de Definições para Sepse e Choque Séptico (Sepsis-3), que definiu sepse como "disfunção orgânica com risco de vida causada por uma resposta desregulada do hospedeiro à infecção" e choque séptico como "um subconjunto de sepse em que anormalidades circulatórias e celulares/metabólicas subjacentes são profundas o suficiente para aumentar substancialmente a mortalidade" (Singer et al., 2016). Além disso, também foi incluído como critério principal para diagnóstico de sepse o uso do escore SOFA (Sequential Organ Failure Assessment). O diagnóstico de sepse pode ser reconhecido por uma alteração na pontuação SOFA total ≥2 pontos, resultante da infecção, seguindo a Figura 1.

Tabela 1.0- Pontuação de avaliação de falha de órgão sequencial [relacionada à sepse]. Critério SOFA (originally the Sepsis-related Organ Failure Assessment) predispõe pontuação de 0-4.

SISTEMAS/ORGÃO	PONTUAÇÃO				
	0	1	2	3	4
RESPIRAÇÃO					
PaO_2/FiO_2 (mmHg)	≥400	<400	<300	<200	<100
CARDIOVASCULAR					
Doses de PAM (mmHg) e catecolaminas [b] são necessárias	PAM ≥ 70	MAPA < 70	Dopamina <5 ou dobutamina (qualquer dose)	Dopamina 5,1–15 ou epinefrina ≤0,1 ou norepinefrina ≤0,1	Dopamina >15 ou epinefrina >0,1 ou norepinefrina >0,1
COAGULAÇÃO					
Plaquetas ($\times 10^3/\mu L$)	≥150	<150	<100	<50	<20
FIGADO					
Bilirrubina (mg/dL)	<1,2	1,2–1,9	2,0–5,9	6,0–11,9	>12,0
NERVOSO					
Escala de coma de Glasgow	15	13–14	10–12	6–9	<6
RENAL					
Creatinina (mg/dL)	<1,2	1,2–1,9	2,0–3,4	3,5–4,9	>5,0
RENAL					
Débito urinário (mL/d)				<500	<200

Figura 1. a- Adaptado de Singer et al., 2016, b- As doses de catecolaminas são calculadas como µg/kg/min durante pelo menos 1h. Abreviaturas: PaO2 = pressão parcial de oxigênio; FiO2 = fração inspirada de oxigênio; PAM = pressão arterial média.

Para evitar atraso no tratamento e agilizar o diagnóstico de pacientes em leitos hospitalares ou internações de urgência, foi desenvolvido o Quick SOFA (qSOFA), uma versão simplificada do escore SOFA que estabelece critérios básicos para o diagnóstico de pacientes adultos. Os critérios para o qSOFA são: frequência respiratória ≥22/min, mentalidade alterada e pressão arterial sistólica ≤100 mmHg. É importante ressaltar que qSOFA não define a sepse, mas possibilita a detecção rápida de pacientes com potencial risco de sepse, uma vez que sinaliza um risco elevado de piora clínica.

2.1.2. Tratamento

O tratamento inicial é feito de forma empírica com o uso de antimicrobianos de amplo espectro, com o objetivo de eliminar os possíveis patógenos causadores da infecção, seguido da reposição de fluidos (cristaloides) e administração de agentes vasoativos (noradrenalina), a fim de estabilizar a pressão arterial e aumentar as chances de sobrevivência do paciente em situações mais críticas (Evans et al., 2021; Guarino et al., 2023; Vincent, 2022). Dito isso, o manejo de pacientes sépticos envolve três aspectos principais: o controle da infecção subjacente, a estabilização hemodinâmica e a modulação da resposta do hospedeiro. Guias como o "Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021" e o "Guia Prático de Terapia Antimicrobiana na Sepse - Instituto Latino Americano de Sepse (ILAS)" oferecem recomendações abrangentes para clínicos no manejo da sepse, auxiliando na padronização do tratamento, com orientações específicas para administração de antimicrobianos e terapias de suporte, além de abordar estratégias para ajustes do tratamento com base nos resultados microbiológicos e na evolução clínica do paciente.

As orientações do ILAS destacam a importância da administração imediata de antimicrobianos, por via endovenosa, o mais rápido possível, preferencialmente dentro da primeira hora após o diagnóstico. Concomitante a isso, devem ser coletadas amostras microbiológicas para identificar o patógeno e direcionar a terapia, entretanto, o início do tratamento não deve ser atrasado enquanto se aguarda pelos resultados microbiológicos. Além disso, pacientes com sinais de hipoperfusão devem ser submetidos à ressuscitação hemodinâmica, utilizando cristaloides para restaurar a perfusão e estabilizar os parâmetros hemodinâmicos. As diretrizes do ILAS também solicitam atenção aos fatores de risco para patógenos resistentes, como internações hospitalares recentes, uso de dispositivos invasivos e exposição prévia a antibióticos, visto que pacientes com histórico de infecções hospitalares ou exposição prolongada a antibióticos apresentam maior risco de infecções por microrganismos multirresistentes, e portanto, nesses casos, pode ser necessária a combinação de terapias antimicrobianas específicas. Vale ressaltar que apenas parte das orientações são apresentada aqui, o guia atualizado aborda de forma completa os fatores a serem considerados no manejo da sepse, com o auxílio de tabelas de terapia antimicrobiana

otimizada para adultos e crianças, incorporando indicação de doses, diluições e interações medicamentosas. As orientações estão disponíveis de forma integral no Guia Prático de Terapia Antimicrobiana na Sepse (ILAS, 2021).

As diretrizes internacionais para o manejo da sepse e do choque séptico de 2021 (Evans et al., 2021), assim como o ILAS, destacam a importância da identificação antecipada e o tratamento adequado nas horas iniciais após o desenvolvimento da sepse como melhora do prognóstico do paciente. Porém, diferente de outras recomendações, o guia em questão orienta suspender o uso de antimicrobianos de forma empírica, em caso de infecção não confirmada, e reavaliar/buscar continuamente diagnósticos alternativos, para maximizar as chances de pacientes infectados receberem terapia antimicrobiana otimizada e pacientes não infectados evitarem terapia não recomendada. Vale lembrar que as instruções disponíveis nesses documentos não substituem a capacidade de tomada de decisão do clínico, elas servem como orientações para os profissionais que atuam em leitos hospitalares no tratamento dessa condição tão desafiadora que é a sepse.

2.1.3. Imunossupressão induzida por sepse

Pacientes com sepse apresentam diferentes estágios da disfunção orgânica. Inicialmente, a sepse desencadeia uma resposta inflamatória intensa mediada pela liberação maciça de citocinas pró-inflamatórias, conhecida como tempestade de citocinas, podendo resultar em lesões teciduais e disfunção de múltiplos órgãos (MODS) (Carcillo & Shakoory, 2024). No entanto, ela também é marcada por um estágio de imunossupressão, caracterizado por liberação de citocinas anti-inflamatórias, alterações metabólicas nos leucócitos, morte de células efetoras e alterações na expressão genética que comprometem as funções das células imunológicas (Torres et al., 2022). A continuidade deste cenário de imunossupressão predispõe os pacientes a infecções oportunistas e complicações graves, aumentando a morbimortalidade a longo prazo.

Torres e colaboradores (Torres et al., 2022) fornecem uma visão geral dos mecanismos envolvidos na imunosupressão induzida por sepse, representados na Figura 2. O início da sepse é desencadeado pela interação entre receptores de reconhecimento de padrão (PRRs) e padrões moleculares associados a patógenos

(PAMPs) ou associados a danos (DAMPs). A interação ativa o sistema imunológico, promove o recrutamento de leucócitos e liberação de mediadores inflamatórios que, em pacientes sépticos, podem ser excessivos, contribuindo para os agravamentos citados anteriormente. Além disso, alterações transcriptômicas, modificações epigenéticas e disfunções metabólicas nas células imunes são observadas durante esse processo. Simultaneamente, são ativados mecanismos anti-inflamatórios, muitas vezes como uma resposta contra a inflamação inicial. Esta etapa é marcada por anergia celular, apoptose e/ou prejuízo na função efetora de linfócitos T e B, com aumento na expressão de moléculas inibidoras de checkpoints (ex: PD-1, PD-L1, CTLA-4), reprogramação de monócitos e macrófagos por alterações epigenéticas, entre outras disfunções. Além disso, alguns agentes administrados em pacientes sépticos podem agravar a imunossupressão, como a norepinefrina, utilizada para estabilização hemodinâmica, e hidrocortisona, para controle da inflamação. Esses agentes têm sido associados à maior persistência desse estado imunossuprimido.

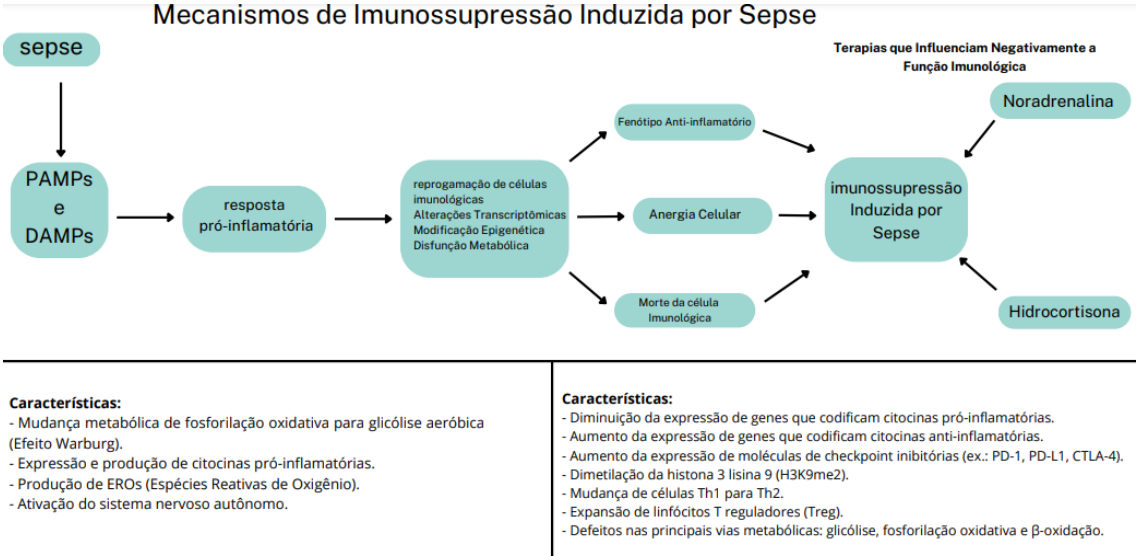


Figura 2. Mecanismos de imunossupressão induzida pela sepse. Adaptada de Torres et al., 2022.

2.2. β-GLUCANAS

2.2.1. Primeiros relatos

É provável que o uso de polissacarídeos como agentes imunomoduladores date da década de 1940, com os estudos de Shear e colaboradores (Shear et al., 1943). Neste estudo foi descrito uma substância, oriunda de culturas de *Serratia marcescens*, e que apresentava atividade antitumoral. Essa substância veio a ser conhecida como polissacarídeo de Shear. Alguns anos depois, Srivastava e colaboradores (Srivastava et al., 1962), caracterizaram o polissacarídeo de Shear como uma mistura polissacarídeos com a cadeia principal consistindo de unidades de d-glicose e d-manose unidas por ligações glicosídicas (1→3), com a ressalva de que não fora descartado completamente a possibilidade da presença de uma mistura de uma glucana e uma manana no complexo polissacarídeo celular.

Com o passar do tempo, novos agentes imunomoduladores foram surgindo, e entre eles as β-glucanas. O estudo com β-glucanas teve início nos Estados Unidos da América (EUA), com base nos estudos do zymosan, e no Japão, com o consumo de cogumelos medicinais. Nos EUA, Pillemer & Ecker (Pillemer & Ecker, 1941) investigaram o zymosan, um polissacarideo oriundo de isolados da parede celular de *Saccharomyces cerevisiae*. Outros estudos que vieram posteriormente, como o de Di Luzio & Riggi (Di Luzio & Riggi, 1970) e Williams e colaboradores (Williams et al., 1980) também são considerados pioneiros e foram de extrema importância no estudo com β-glucanas. No Japão, o surgimento de β-glucanas estava fortemente associado a medicina tradicional, com o consumo de cogumelos medicinais, como shiitake, maitake, entre outros. Chihara e colaboradores (Chihara et al., 1969), isolaram o lentinan, uma β-glucana oriunda do cogumenlo shiitake (*Lentinula edodes*) que apresentou atividade antitumoral em camundongos inoculados com sarcoma. Os estudos em questão serviram de base para investigações posteriores que levaram à caracterização de β-glucanas. Portanto, é possível que estes sejam os primeiros relatos documentados sobre os efeitos associados a esses polissacarídeos.

2.2.2. Caracterização e Fontes das β-glucanas

β-glucanas são polissacarídeos formados por monômeros de glicose unidos por ligações glicosídicas β (beta) (Synytsya & Novak, 2014). A estrutura linear central é formada por monômeros de β-1,3 glicose, e devido à possibilidade de variações no seu comprimento de ramificação principal, ligações e padrões de ramificação, uma diversidade de moléculas de β-glucanas são formadas, desempenhando diferentes atividades biológicas (Murphy et al., 2021). Este grupo de moléculas geralmente é obtido a partir da parede celular de fungos, leveduras, cereais e bactérias (Kaur et al., 2020), e dada a diversidade estrutural de β-glucanas, as classificações são amplamente baseadas na estrutura, pois os β-glucanas de fonte cereal apresentam estrutura diferente daqueles de fontes não cereal.

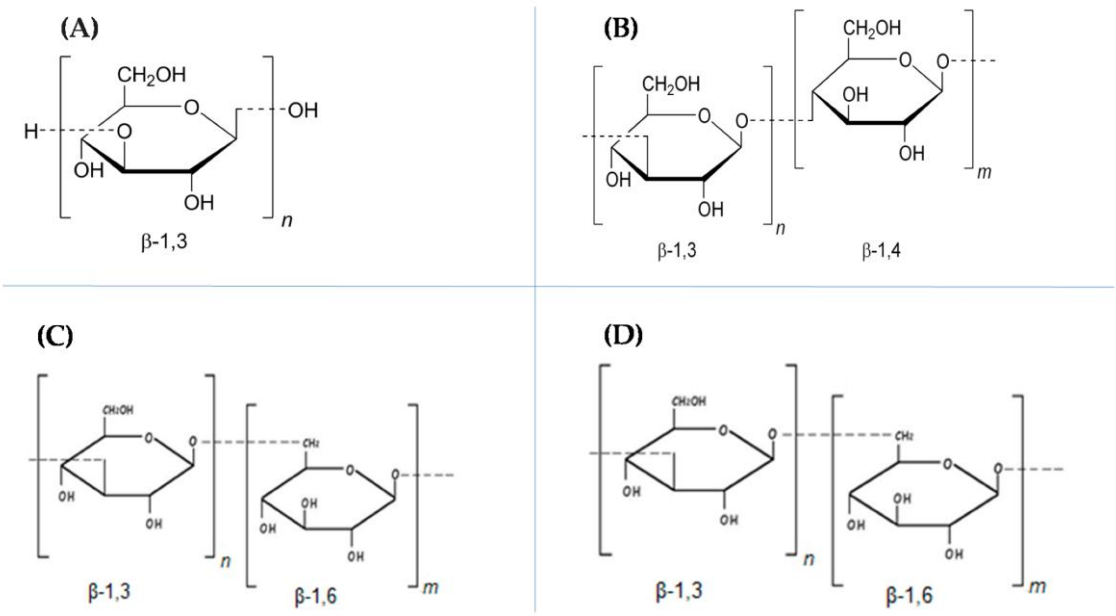


Figura 3. A estrutura química do β-glucano de diferentes fontes. (A) β-glucano de bactérias; (B) β-glucano de cereais ou líquenes; (C) β-glucano de algas marinhas; (D) β-glucano de fungo ou levedura; (Kaur et al., 2020)

Conforme demonstrado na Figura 3 por Kaur e colaboradores (Kaur et al., 2020), as β-glucanas diferem em sua estrutura química dependendo da fonte: Cereais, como a aveia e cevada, possuem β-glucanas compostas principalmente por ligações β(1→3) e β(1→4), sem a presença de ligações β(1→6) (Gupta et al., 2010; Johansson et al., 2000). Leveduras, como *Saccharomyces cerevisiae* apresentam β-glucanas com

cadeias lineares β(1→3) e longas ramificações unidas por ligações β(1→6) (Manners et al., 1973). Fungos contêm β-glucanas com cadeias lineares de β(1→3) e curtas ramificações unidas por ligações β(1→6) (Han et al., 2008; Manners et al., 1973). Bactérias, como *Agrobacterium biobaris*, possuem β-glucanas com cadeias lineares β(1→3)-D-glucano, sem ramificações (McIntosh et al., 2005). E algas marinhas, como os kelps marrons do gênero *Laminaria*, podem apresentar β-glucanas de cadeias β(1→3) lineares ou com ramificações β(1→6), dependendo da espécie (Bonfim-Mendonça et al., 2017; Teas, 1982).

2.2.3. Ligantes, Sinalização e Atividade Biológica

No sistema imunológico, as β-glucanas interagem com receptores de reconhecimento de padrão (PRR) específicos, como o receptor de complemento 3 (CR3) (Xia et al., 1999), a Dectina-1 (Brown et al., 2003; Herre et al., 2004) e TLR-2/6 (Schorey & Lawrence, 2008), induzindo resposta imunológica protetora. A Dectina-1 é um receptor transmembrana tipo II, que compõe a família das lectinas tipo-c (CLRs) (Bauer & Steinle, 2017). Inicialmente, esse receptor foi descrito como o principal receptor de β-glucanas em macrófagos (Brown et al., 2003; Brown et al., 2002), entretanto existem uma série de ligantes endógenos e exógenos com relevância fisiológica em diferentes patologias, conforme demostrado na Figura 4.

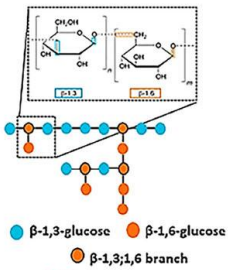
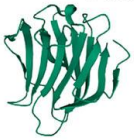
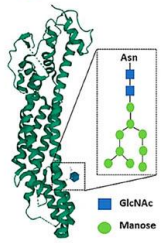
Ligand	Structure	Pathology	Recognized by	Physiological relevance	References
β-glucans		Microbial infections	Neutrophils Macrophages Monocytes Dendritic cells Keratinocytes Epithelial cells	Proinflammatory: IL-1β, IL-6, IL-23, TNF-α and ROS production. Phagocytosis of pathogens.	(20, 24–28)
Galectin-9		Autoimmune diseases	Neutrophils Macrophages Microglia Dendritic cells	Anti-inflammatory: tolerance and induction of oncostatin M overexpression.	(32)
		Cancer	Macrophages	Immune tolerance: low MHC-II, iNOS and TNF-α, high CD206 expression. Tumor progression.	(33)
Annexins		Autoimmune diseases and aging	Dendritic cells	Immune tolerance: reduced ROS production, dampening CD80 and CD86 production.	(34)
Vimentin		Atherosclerosis	Myeloid cells	Proinflammatory.	(35)
		Ischemia/reperfusion	Macrophages	Proinflammatory.	(36)
			Neutrophils	M1-macrophage recruitment, myocardial injury, and apoptosis.	
		Obesity	Macrophages	Proinflammatory: insulin resistance.	(37)
Tropomyosin		Allergy	Epithelial cells	Immune tolerance: reduced allergic symptoms and IL-33 dampening.	(38)
N-glycan		Cancer	Dendritic cells Macrophages	Proinflammatory: Anti-tumor response *via* induction of natural killer cells cytolytic capacity.	(39, 40)

Figura 4. Ligantes de Dectina-1 (Mata-Martínez et al., 2022).

Além de β-1,3-glucanas com ramificação β-1,6 constituírem os principais ligantes da Dectina-1, outras moléculas, tais como: Galectina-9, Annexinas, Vimentina, Tropomiosina e N-glicano, se ligam a esse receptor, participando do desenvolvimento de patologias como doenças autoimunes, câncer, aterosclerose, obesidade, alergias entre outras (Bode et al., 2019; Gour et al., 2018; Weyd et al., 2013; Yang et al., 2021; Yang et al., 2008).

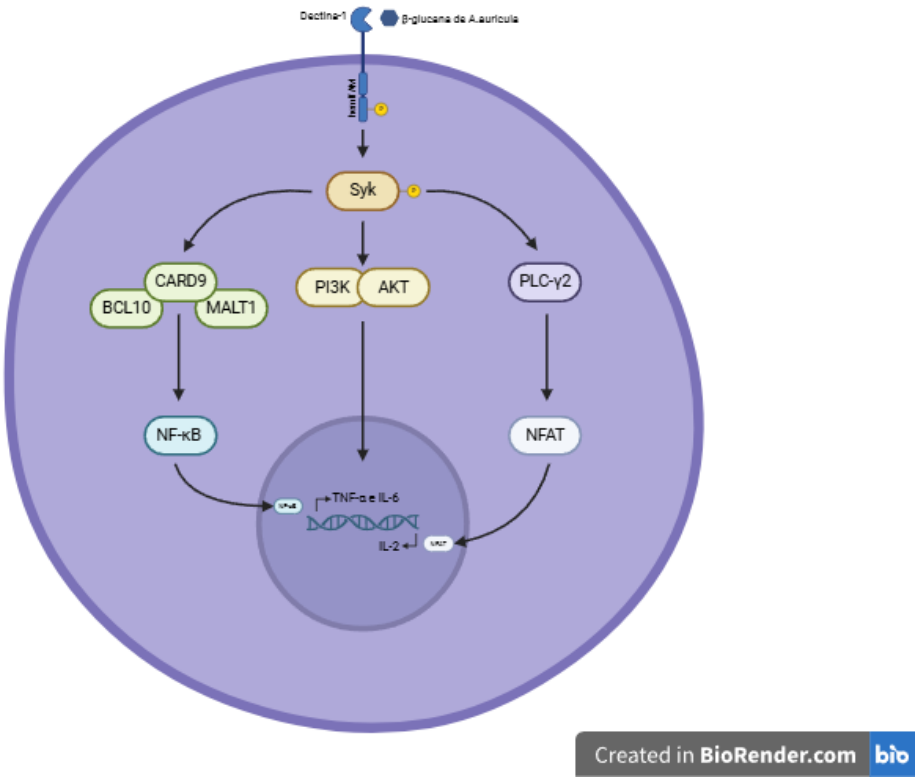


Figura 5. Sinalização da Dectina-1 mediante a ligação por β-glucana. A β-glucana é reconhecida por dectina-1, que então é fosforilada em seu domínio hemITAM, facilitando o encaixe para Syk. A cascata de sinalização de Syk engloba o complexo proteico CARD9/BCL10/MALT1 e PI3K/AKT, resultando na ativação de NF-κB e produção de TNF-α e IL-6. E por meio de PLC-γ2, resulta na ativação de NFAT, impulsionando a produção de IL-2.

Após a ligação de β-glucanas, a Dectina-1 desencadeia uma cascata de sinalização ativando vias dependentes de Syk, que incluem o complexo CARD9/BCL10/MALT1 e PIK3/AKT, resultando na ativação de NF-κB e produção de citocinas pró-inflamatórias (como TNF-α e IL-6). Além disso, a cascata de sinalização leva à ativação de NFAT, culminando na produção de IL-2 (Figura 5) (Mata-Martínez et al., 2022). Adicionalmente, o estimulo inicial induz mudanças epigenéticas, possibilitando maior acessibilidade à cromatina e as histonas, como a H3K27ac, H3K4me1 e H3K4me3 (Wang et al., 2024), resultando em estado de maior responsividade de longo prazo em células imunes inatas, conhecido como imunidade treinada — um estado de memória funcional da imunidade inata que amplifica respostas a estímulos secundários (Cheng et al., 2024). No contexto da sepse, a modulação da via da Dectina-1 por β-glucanas pode equilibrar a resposta imune, evitando sintomatologia desencadeadas pela sepse, isso ocorre porque a sinalização via Dectina-1 pode regular a produção de mediadores inflamatórios (Wang et al.,

2024). Dessa forma, a ativação controlada desse receptor por β-glucanas, como as derivadas de *A. auricula*, pode representar uma estratégia terapêutica promissora para modular a resposta imune em condições críticas, como a sepse.

Além dos efeitos biológicos descritos anteriormente, as β-glucanas possuem também efeitos como: redução nos níveis de colesterol (Behall et al., 1997; Keogh et al., 2003; Whitehead et al., 2014), efeito antitumoral (Akramiene et al., 2007; Lemieszek & Rzeski, 2012) e efeito imunomodulador. Um estudo demonstrou que uma β-glucana de levedura melhora as respostas antitumorais das células NK (natural killers) contra câncer, ao modular o perfil fenotípico de macrófagos (Zhu et al., 2023). Além disso, também já foi descrito o papel de β-glucanas na defesa contra infecções bacterianas: Moorlag e colaboradores (Moorlag et al., 2020), demonstraram que o tratamento com β-glucana aumenta a atividade antimicrobiana de monócitos humanos e aumenta a resistência de camundongos à infecção por *Mycobacterium tuberculosis*. Ainda no contexto de infecções, a administração de glucanas também demonstrou efeitos protetores contra *Leishmania major (Al Tuwaijri et al., 1987)*, *Staphylococcus aureus (Liang et al., 1998)*, *Trypanosoma cruzi* (Williams et al., 1989) e outros agentes etiológicos.

Esta vasta variedade de funções biológicas evidencia a importância do estudo com β-glucanas em áreas como a saúde humana e a biotecnologia. Além disso, a capacidade das β-glucanas de interagir com PRRs e modular a resposta imunológica faz delas potenciais agentes terapêuticos no desenvolvimento de novas estratégias para condições como a sepse, que requer uma abordagem capaz de modular a resposta imunológica do paciente.

3. OBJETIVOS

3.1. GERAL

Investigar o efeito do tratamento com β-glucana de *Auricularia auricula* em modelo de infecção sistêmica do tipo sepse letal polimicrobiana.

3.1.1. ESPECÍFICOS

- Apurar o efeito do tratamento profilático de β-glucana sobre a migração e proliferação de leucócitos;
- Quantificar a celularidade nos órgãos linfoides e no foco infeccioso;
- Investigar o perfil de citocinas em animais com ou sem infecção;
- Averiguar as possíveis alterações histopatológicas no pulmão;
- Investigar o efeito do tratamento profilático sobre a disseminação bacteriana *in vivo*;
- Acompanhar a sobrevida dos animais tratados com a β-glucana;

4. CAPÍTULOS

Os dados obtidos neste estudo embasaram a construção de dois produtos:

I. Artigo de revisão publicado na revista Journal of Immunology Research, intitulado: "Glucans: A Therapeutic Alternative for Sepsis Treatment". Fator de Impacto do Periódico em 2023 (Clarivate): 3.5. CiteScore em 2023 (Scopus): 6.9. Qualis A3 (Quadriênio 2017-2020).

II. Artigo em processo de submissão, na revista International Journal of Food Sciences and Nutrition, intitulado: "*Auricularia auricula's* polysaccharides confers protection in murine Sepsis via Immune Modulation". Fator de Impacto do Periódico em 2024: 3.3. CiteScore em 2024 (Scopus): 7.1. Qualis A2 (Quadriênio 2017-2020).

4.1. REVISÃO

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WILEY

Review Article

Glucans: A Therapeutic Alternative for Sepsis Treatment

Jesse P. M. Viana[1], **Fernanda F. Costa**[2], **Tatielle G. Dias**[3], **Priscila M. Mendes**[3], **Gabriel B. Copeland**[4], **Willian S. Nascimento**[4], **Sofia S. N. Mendes**[4], **Isabella F. S. Figueiredo**[5], **Elizabeth S. Fernandes**[5], **Anamelia L. Bocca**[1,6] and **Márcia C. G. Maciel**[1,2,3]

[1]*Departamento de Biologia Celular, Instituto de Ciências Biológicas, Programa de Pós Graduação em Ciências Biológicas (Biologia Molecular), Laboratório de Imunologia Aplicada, Universidade de Brasília (UnB), Brasília, Brazil*
[2]*Programa de Pós-graduação em Saúde e Tecnologia, Universidade Federal do Maranhão, São Luís, Brazil*
[3]*Programa de Pós-graduação em Ciências da Saúde, Universidade Federal do Maranhão, São Luís, Brazil*
[4]*Laboratório de Imunologia Aplicada, Universidade de Brasília (UnB), Brasília, Brazil*
[5]*Programa de Pós-graduação em Biotecnologia Aplicada à Saúde da Criança e do Adolescente—Faculdades Pequeno Príncipe, Instituto de Pesquisa Pelé Pequeno Príncipe, Curitiba, Brazil*
[6]*Plataforma Bi-Institucional de Pesquisa Translacional—Fiocruz/SP, São Paulo, Brazil*

Correspondence should be addressed to Márcia C. G. Maciel; macielmcg@gmail.com

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Sepsis treatment is a challenging condition due to its complexity, which involves host inflammatory responses to a severe and potentially fatal infection, associated with organ dysfunction. The aim of this study was to analyze the scientific literature on the immunomodulatory effects of glucans in a murine model of systemic infection induced by cecal ligation and puncture. This study comprises an integrative literature review based on systematic steps, with searches carried out in the PubMed, ScienceDirect, Scopus, Web of Science, and Embase databases. In most studies, the main type of glucan investigated was β-glucan, at 50 mg/kg, and a reduction of inflammatory responses was identified, minimizing the occurrence of tissue damage leading to increased animal survival. Based on the data obtained and discussed in this review, glucans represent a promising biotechnological alternative to modulate the immune response and could potentially be used in the clinical management of septic individuals.

1. Introduction

Sepsis is a global public health problem, causing an estimated 11 million deaths annually [1]. Despite a decrease in its incidence and mortality rates, sepsis remains one of the main contributors to decline in health on a worldwide scale, affecting almost 50 million individuals globally [2]. Sepsis can develop from bacterial, viral, fungal, or parasitic infections [3], and it is characterized by a deregulated inflammatory response to an infection, culminating in multiple organ dysfunctions. Currently, the most widely used therapeutic interventions for sepsis aim to controlling the infectious agent through the administration of antimicrobials, combined with supportive approaches, such as fluid resuscitation. Individual immunomodulatory treatments have been used and were able to reduce mortality in some but not all patients including IFN-γ, GM-CSF, IL-7, PD-1 antagonists, cannabinoids, thymosin α-1, PMX-HP, IL-6 inhibitors, and IL-1β antagonists [4]. Thus, there is a constant search for immunomodulators motivated by the lack of specific drugs to treat patients with sepsis. In this context, investigations of new therapeutic strategies, such as β-glucan, a natural compound that is abundant in nature and has immunomodulatory properties, remain a focus [5, 6].

Glucans are a group of natural polysaccharides with wide structural diversity, found in various sources, such as cereals, mushrooms, and yeasts. Their consumption has been linked to anticancer [7], antidiabetic [8], and immune system stimulatory [9] activities, as well as to the treatment of infections [10] and sepsis [11]. β-Glucans are structurally described as D-glucose polymers joined via β-glycosidic bonds [12].

Despite the diversity of available sources for the extraction of β-glucans, the most widely investigated are the fruiting bodies of different types of mushrooms, notably yielding $(1 \rightarrow 3)$ and $(1 \rightarrow 6)$-β-glucans [13]. The immunomodulatory activity of glucans occurs mainly through the stimulation of immune cells *via* pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), complement receptor-3 (C3), and receptor for glucans on dendritic cells (dectin-1) [14].

Dectin-1 is the most well-studied receptor for β-glucans, originally described as the "β-glucan receptor expressed on myeloid cells, playing essential roles in immune responses against fungi" [15]. It is a glycoprotein that has a carbohydrate recognition domain, allowing it to identify glucans [16]. This receptor is mainly expressed on cells of myeloid origin, such as dendritic cells, macrophages, and neutrophils, although its expression has been also observed on cells of lymphoid origin, such as T and B lymphocytes [17]. Ni et al. [18] demonstrated that the interaction of a monoclonal antibody (mAb) anti-dectin-1 with dectin-1 receptors induces dendritic cells to stimulate $CD8^+$ T lymphocyte responses. As a result of this interaction, a positive regulation of costimulatory molecules and secretion of cytokines and chemokines, leading to increased antigen presentation, activation, and expansion of $CD8^+$ T cells, were observed [14]. Similar effects on $CD8^+$ T cells were attributed to β-glucans [19].

Although the biological effects of β-glucan exposure are not yet fully understood, the literature suggests these polysaccharides are strong stimulators of the immune system. Recently, β-glucans were found to have the ability to induce trained immunity. Indeed, this group of molecules can stimulate innate immune responses by acting on cells such as neutrophils, monocytes, and macrophages, resulting in reprogramming of their metabolic and epigenetic states. Once the body is exposed to a second stimulus with β-glucans or other infectious challenges, an amplified innate immune response results, translating into the enhanced recruitment of immune system cells. This contributes to a more effective eradication of invading pathogens and consequently improves the ability of the body to fight the infection and survive [5].

In summary, the study of glucans has become increasingly important, especially because of their immunomodulatory effects. Research has demonstrated their protective properties against the lung damage caused by sepsis, evidenced by a reduction in the percentages of neutrophils and lymphocytes in the bronchoalveolar lavage [20], and increased survival rates in septic animals [21].

The aim of this integrative review is to describe the immunomodulatory potential of glucan treatment by focusing in a murine model of sepsis induced by cecal ligation and puncture. At the end of this review, we provide a comprehensive understanding of the biotechnological potential of glucans from a clinical perspective in order to provide with information for both healthcare professionals treating patients with sepsis and researchers seeking better therapeutic approaches.

2. Materials and Methods

An integrative literature review was developed to provide an extensive analysis of the potential of glucans to treat sepsis. The steps followed to construct this review were as follows: identification of the topic and elaboration of the guiding research question; selection and extraction of data; categorization of studies; data analysis; synthesis of results; and presentation of the integrative review [22]. We developed the research strategy following the PICO strategy, using the guidelines of the Joanna Briggs Institute (JBI), an acronym where "P" represents the population (glucan), "I" the interest (cecal ligation and puncture), and "CO" the context (sepsis). We thus obtained the following guiding question, from which we selected keywords for the database search: "What is the scientific evidence related to the effects of glucans in a model of cecal ligation and puncture in septic conditions?"

To find relevant studies, five databases were searched, PubMed, ScienceDirect, Scopus, Web of Science, and Embase; these were chosen because of their wide coverage and importance in the research area. The searches were conducted using keywords and descriptors controlled by Medical Subject Headings (MeSH) and the noncontrolled terms "glucan," "sepsis," and "cecal ligation and puncture," along with the Boolean operators "AND" and "OR," adapting them to each specific database when necessary.

The Rayyan platform was used to organize the articles obtained and to help excluding duplicated articles and reviews [23]. The inclusion criteria considered only studies that addressed the effects of β-glucans in sepsis models of cecal ligation and puncture. Studies published in English and available in full were included. Articles that did not include all the proposed descriptors were duplicates and narrative, integrative, or systematic reviews, and meta-analyses were excluded from the current review, as well as theses, dissertations, and unavailable articles. We used an adaptation of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart to describe the stages of article selection [24]. The data were collected based on the following information: year of publication, title, objective, type of study, type of glucan, dose, route of administration, treatment schedule, and conclusions. After extracting the data, the immunomodulatory and therapeutic potential of β-glucans was considered for a clearer understanding of the topic.

3. Results

Following the searches in the databases, 476 studies were identified. Of these, 76 duplicates were excluded, leaving 400 studies. Then, 372 studies were excluded either for having missing descriptors or being review articles. After screening, 28 studies were selected to be read in full, with 11 of them being removed for being either out of context or unavailable in full. Therefore, 17 articles were included in this review (Figure 1).

To clarify the immune mechanisms associated with glucan treatment, the most relevant aspects observed in the studies were summarized (Table 1). Based on the data extracted after reading the selected articles and after analysis of Table 1, a figure was generated in order to illustrate the mechanisms of action of glucans in the immune and inflammatory responses (Figure 2).

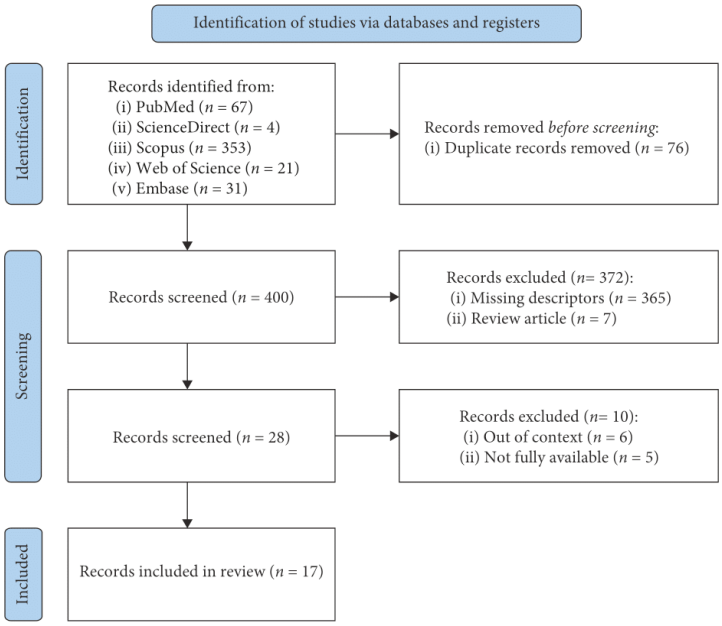

Figure 1: Article selection flowchart (adapted from PRISMA 2020 flowchart).

According to the selected studies, the modulatory actions of the glucans are mainly due to their abilities to reduce proinflammatory cytokines and the activity of enzymes directly linked to the inflammatory process including myeloperoxidase, preventing multiple organ failure and increasing the survival of septic animals (Figure 2).

4. Discussion

β-Glucan was the main type of glucan studied among the selected articles (88.2%). β-Glucans are polysaccharides widely present in the cell wall structure of various plants and microorganisms, and they have a high affinity for receptors linked to the immune response in mammalians. These macromolecules are formed through the union of smaller monosaccharides, *via* glycosidic bonds, and play a fundamental role in the structure of the cell wall of the organisms in which they are present. Glucans are subject to the action of various enzymes responsible for generating the bonds that join the smaller units of monosaccharides and that participate in the construction and conformation of the polysaccharide [39]. This is an important factor since various studies have described how the chemical structure of glucans is directly related to their biological activity [40].

The characteristic glycosidic bonds of these molecules are related to their origin and bioactivity. The β-glucans isolated from fungi have a $1 \rightarrow 3$ bond with branches at $1 \rightarrow 6$, whereas those isolated from cereals, such as wheat, oats, and rice, have bonds at $1 \rightarrow 3$ and $1 \rightarrow 4$ and have long linear chains [41]. The positions of these bonds have a direct effect on the biological activity of β-glucans, with the $1 \rightarrow 3$ bond being most associated with strong immune responses, whereas the $1 \rightarrow 4$ bond is associated with a lower or no immune responses [40]. Among the selected articles, six [26, 27, 28, 29, 30, 35] specified the use of $(1 \rightarrow 3)$-β-glucan with associated increases in IL-10 [26] and IL-6 [26], reductions of TNF-α [27, 35] and IL-6 [27, 35], and increased survival rates [27, 30, 34, 35]. These effects prevented cardiac dysfunction and inhibited cardiomyocyte apoptosis [28], reducing TLR4 expression [29], NF-κB activity [29], and cytoplasmic levels of HMGB1 [29], in addition to decreasing myeloid suppressor cells [35]. Meanwhile, two studies [36, 37] reported that $1 \rightarrow 3\text{-}1 \rightarrow 6$-$\beta$-glucans are associated with a reduction in zinc and copper levels [36] in the liver, lung, kidney, heart, and diaphragm; a reduction in TNF-α [37] and MDA [37] levels; and a reduction of neutrophil infiltration in all tissues investigated such as the lung, heart, liver, kidney, diaphragm, and brain [37].

Among the articles included in the review, only one evaluated the activity of α-glucans in a model of sepsis [35]. The α-glucan YCP isolated from the fungus *Phoma herbarum* showed significant immunomodulatory activities, reducing inflammatory cytokines, such as IL-6 and serum TNF-α, in septic mice and regulating the frequency of myeloid suppressor cells [42]. The α-glucans synthesized by fungi have complex structures and different glycosidic, and although there are more studies on the bioactivity of β-glucans, α-glucans isolated from the walls of fungi have shown immunomodulatory activity similar to that of β-glucans, as well as antitumor activity after modifications to improve their solubility [43].

Table 1: Distribution of selected studies according to formulation, type of study, type and dose/concentration of the glucan, route of administration, treatment schedule, immunological activity, and references.

Source	Glucan type	Type of study	Dosage/ concentration	Route of administration	Treatment schedule	Immunological activity	Ref.
Microorganism: yeast, *Saccharomyces cerevisiae*	β-Glucan (microparticulate form, suspended with saline, 50 mg kg^{-1}, Immunex R, M.N.C.)	*In vivo* (*rats: Wistar albino rats of both sexes*)	50 mg (kg)	GIG	One time day/for 10 days + 30 min before CLP	↓ TNF-α, ↓ IL-6, ↑ IL-10 soro ↑ superoxide dismutase (SOD), and catalase (CAT) in the liver tissue ↓ malondialdehyde (MDA) in the liver	[25]
Plant: cereal and barley	β-D-Glucan (Sigma, St. Louis, MO, USA)	*In vivo* (*rats: male Wistar albino*)	2 mg (kg)	IP	One time post-CLP surgery	↓ Pulmonary MPO; ↑ leukocytes in the blood, ↑ monocytes in the blood; ↓ neutrophils in the blood; ↓ neutrophils and lymphocytes in the BAL, ↑ monocytes in the BAL; ↑ serum IL-6, ↓ alveolar hemorrhage	[19]
Plant: cereal and barley	β-D-Glucan (Sigma Chemical Company, St Louis, MO)	*In vivo* (*rats: male Wistar*)	2 mg (kg)	IP	Just after CLP with an additional one injection at 4 hr after CLP	↑ Survival rate to 63% ↓ the levels of TNF-α, IL-1β, and IL-6 ↓ lung MPO ↓ lung ICAM-1	[20]
Not specified	Pachyman (Associates of Cape Cod, Falmouth, MA, USA)	*In vivo* (*mice: female FcGRIIb −/− C57BL/6*)	50 mg (kg)	IV	3 and 6 hr post-CLP surgery	↑ IL-10 ↑ IL-6	[26]
Microorganism: yeast, *S. cerevisiae*, and fungi, *Sclerotium glucanicum*	(1 → 3)-β-D-Glucans (glucan phosphate and scleroglucan)	*In vivo* (*mice: male ICR/HSD*)	50 mg (kg)	IP	Pretreatment 1 hr before CLP and posttreatment 15 min after CLP	Pretreatment: glucan phosphate: ↓ liver NF-κB and NF-IL6; ↓ lung NF-κB and NF-IL6; ↑ survival rate by 65%. ↓ hepatic TNF-α and IL-6; ↓ lung TNF-α and IL-6; scleroglucan: ↓ liver NF-κB and NF-IL6; ↓ lung NF-κB and NF-IL6; ↑ increases survival rate in 75% posttreatment: glucan phosphate: ↓ liver NF-κB and NF-IL6; ↓ lung NF-κB and NF-IL6; ↑ survival rate by 65%; ↓ hepatic TNF-α; ↓ lung TNF-α and IL-6	[27]
Microorganism: yeast, *S. cerevisiae*	(1 → 3)-D-Glucan (glucan phosphate)	*In vivo* (*mice: male ICR/HSD*)	40 mg (kg)	IP	1 hr before induction of CLP	↓ Cardiac dysfunction activates the phosphoinositide 3-kinase/Akt pathway; ↓ myocardial MIF expression; ↓ cardiomyocyte apoptosis; prevented the decrease phospho-Akt and phospho-GSK-3β; prevented the decrease in Bcl-2	[28]
Microorganism: yeast, *S. cerevisiae*	(1 → 3)-D-Glucan (glucan phosphate)	*In vivo* (*mice: male ICR/HSD*)	40 mg (kg)	IP	1 hr before induction of CLP (GP foi dissolvido em solução salina)	↓ Translocation of HMGB1 ↓TLR4 expression ↓ myocardium NF-κB	[29]
Microorganism: yeast, *S. cerevisiae*	(1 → 3)-D-Glucan (glucan phosphate)	*In vivo* (*mice: male ICR/HSD*)	50 mg (kg)	IP	1 hr before induction of CLP	↑ Long-term survival (20% vs. 70%) ↓ TLR2/4 gene and TLR4 protein expression	[30]
Microorganism: nonrecombinant yeast strain, *S. cerevisiae*	β-Glucan PGG glucan (Imprime-PGG) (Eagan, MN)	*In vivo* (*mice: male, female, and ovariectomized female CD-1 mice*)	10 mg (kg)	IP	1 hr after induction of CLP	Male: ↑ survival only 24 hr femal: ↑ survival over a 10-day period, ↓ interleukin-6 (IL-6) and IL-10 ↓ CFU in the liver, ovariectomy: abrogated the response to PGG glucan	[31]
Microorganism: yeast, *S. cerevisiae*	(1 → 3)-D-Glucan (glucan phosphate)	*In vivo* (*mice: male ICR/HSD*)	40 mg (kg)	IP	1 hr before induction of CLP	↑ Survival by 60%; ↑ PI3K activity	[32]
Plant: cereal and barley	β-D-Glucan (Sigma Chemical, St. Louis, MO)	*In vivo* (*rats: male Wistar*)	2 mg (kg)	IM	After induction of CLP	↓ Weight loss; ↓ cumulative adhesion score	[33]

Table 1: Continued.

Source	Glucan type	Type of study	Dosage/ concentration	Route of administration	Treatment schedule	Immunological activity	Ref.
Not specified	Glucan-P (Accurate Chemical and Scientific Corp., Westbury, NY) and glucan-F (Laboratory of the Late Dr. N. R. DiLuzio. New Orleans, LA)	*In vivo* (*rats: male Sprague–Dawley*)	10 mg (kg)	IV	Daily for 5 consecutive days	↑ Survival 7-day (glucan-P and/or glucan-F + ampicillin)	[34]
Microorganism: fungi, *P. herbarum*	α-Glucan (YCP) (phoma herbarum YS4108 fungi)	*In vivo* (*mice: male c57bl/6j*)	20 mg (kg)	IP	2 hr before, 4 hr after, and 24 hr after CLP	YCP: ↑ survival from 39% to 72% on d 10 post-CLP ↓ myeloid-derived suppressor cells (MDSCs) in the lungs and livers ↓ STAT3 pathway activation ↑ Interferon regulatory factor-8 (IRF-8) ↓ IL-6 e TNF-α when BM-derived MDSCs were co-cultured with T cells, YCP dose-dependently ↑ Arg-1/iNOS, and activated the NF-κB pathway	[35]
Microorganism: yeast, *S. cerevisiae*	1 → 3−1 → 6-β-D-Glucan (Mustafa Nevzat Company, Turkey. *S. cerevisiae*)	*In vivo* (*rats: albino wistar*)	50 mg (kg)	GIG	Once a day for 10 days	↓ Zinc and copper levels of the liver, lung, kidney, heart, and diaphragm. ↓ Tissue damage	[36]
Microorganism—yeast—*S. cerevisiae*	1 → 3−1 → 6-β-D-glucan (Mustafa Nevzat Company, Turkey. *S. cerevisiae*)	*In vivo* (*rats: Wistar albino rats of both sexes*)	50 mg (kg)	GIG	Once daily for 10 days and 30 min prior to and 6 hr after the CLP	↓TNF-α ↓The MDA levels in the liver, kidney, heart, lung, diaphragm, and brain reversed the GSH level ↓ neutrophil infiltration	[37]
Plant: cereal and barley	β-glucan (CAS: 9041-22-9) Sigma (Shanghai, China)	*In vitro* (*raw 246.7*) *in vivo* (*mice, female ICR*)	30 μg (mL) (in vitro) 500 μg (in vivo)	IP	Twice a week before sacrifice	↓ Lung and liver injury ↓ CFU blood and peritoneum (β-glucan and β-glucan + SPIO); ↓ inflammatory cell infiltration and hyperemia in the alveolar walls (β-glucan, β-glucan + SPIO); ↓ vacuolization and sinusoidal congestion in the liver (β-glucano + SPIO); ↑ TNF-α, IL-1β, and IL-6 mRNA expression	[6]
Plant: cereal and barley	β-Glucan (β-D-glucan, Sigma, St. Louis, MO, USA)	*In vivo* (*rats*)	4 mg (kg)	IP	Following CLP (a) single dose of 4 mg β-glucan (kg)	↓ Plasma MPO/AOPP/MDA ↓ lung MDA ↓ liver MDA	[38]

GIG, intragastric gavage; IP, intraperitonealy; IM, intramuscularly; IV, intravenously; CLP, cecal ligation and puncture; BAL, bronchoalveolar lavage; MIF, migration inhibitory factor; ICAM, intercellular adhesion molecule; MPO, myeloperoxidase; Ref, references.

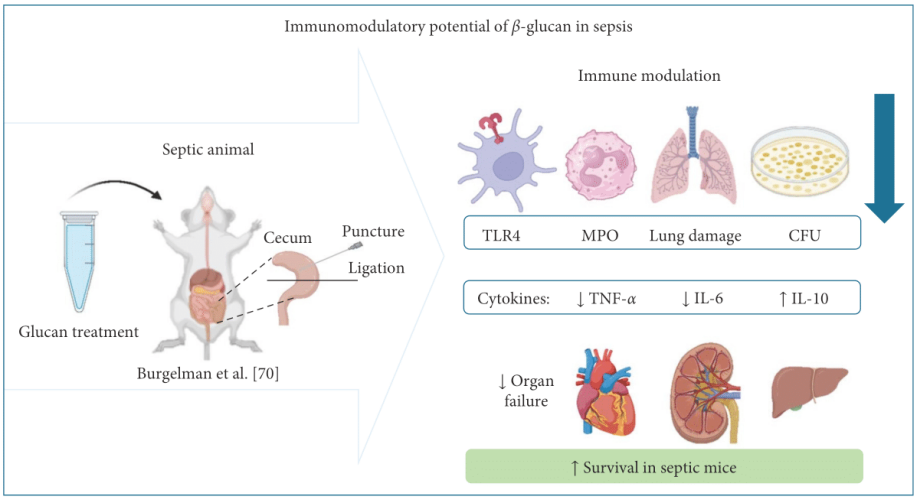

FIGURE 2: Mechanism underlying the protective effects of glucan treatment in cecal ligation and puncture (CLP)-induced infections. Prophylactic and/or therapeutic treatment enables a better immune response from the host in the fight against infection. The mechanism involved in the response involves the modulation of the expression of genes related to mortality, such as TLR-4. The treatment made it possible to reduce the expression of these genes, as well as related proteins, correlating with improved long-term survival. In addition, it allows control of colony-forming units and facilitates a reduction in plasma levels of enzymes that actively participate in the inflammatory process, such as myeloperoxidase (MPO), which is strongly associated with neutrophil infiltration and subsequent tissue damage. The immune response stimulated through treatment with glucan protects the host, not only by increasing its survival rate but also by controlling exacerbated inflammatory response, preventing multiple organ dysfunction syndrome (MODS), highlighting the effectiveness of this natural product as a therapeutic intervention against serious infections.

Similar to that of other polysaccharides and dietary fibers, the mechanisms underlying the effects of β-glucans include increased absorption of nutrients and improvement of the viscosity of intestinal contents. In addition, β-glucans might represent a potential source for fermentation by microorganisms in the small intestine, promoting a prebiotic effect [44]. Arena et al. [45] conducted a study evaluating the symbiotic potential between β-glucans and probiotic strains. Mixtures of probiotic microorganisms and barley β-glucans presented synergistic effects, modulating at transcriptional level, proinflammatory genes encoding TNF-α, NF-κB, IL-8, and IL-1β. In addition, incubation with probiotics significantly increased IL-10 gene expression, leading to an anti-inflammatory effect.

β-Glucans were shown to reduce proinflammatory cytokines, stimulate the release of anti-inflammatory cytokines, and increase the formation of antioxidants [46, 47, 48]. Among the studies analyzed in the review, it was observed that during sepsis, treatment with glucans can reduce inflammatory mediators. With respect to these mediators, of the articles included in this review, 29% reported reductions of TNF-α [20, 25, 27, 35, 37], 29% described attenuated levels of IL-6 [20, 25, 27, 31, 35], and 5.8% diminished production of IL-1β in glucan-treated septic mice. However, in contrast to the aforementioned data, Babayigit [19] showed an increase in IL-6; however, the administered dose of glucan was 2 mg/kg, i.e., lower than that tested in other studies in which decreased IL-6 was observed, such as the study by Newsome et al. [31] that used a dose of 10 mg/kg. Also, Pan et al. [6] described an increase in TNF-α and IL-6 in septic individuals treated with β-glucans (dose of 500 μg). The varied results observed for glucans on IL-6 may be due their different doses in the studies. In addition, Murphy et al. [49] showed that β-glucans from the same *Lentinus edodes* mushroom, one isolated using hot water extraction and the other of commercial origin, had different effects, specifically reducing inflammatory cytokines and reducing macrophage phagocytic activity after stimulation with LPS. This suggests that the source and extraction methods can influence the results.

In this study, four articles [25, 26, 31, 32] addressed the impact of glucans on IL-10 production and its subsequent effects on sepsis outcome. In one of these [25], increased IL-10 reduced TNF-α, IL-6, and IL-1β levels which were noted, whereas in Newsome et al. [31], it was obtained reductions in IL-10 and IL-6. The authors showed a reduction in colony-forming unit (CFUs) and an increase in animal survival.

In addition, certain genes, transcription factors, and receptors related to the inflammatory response were analyzed. NF-κB and nuclear factor interleukin 6 (NF-IL6 or CCAAT enhancer binding protein (C/EBP)) are transcriptional activator proteins that participate in the induction of numerous cellular genes and are intrinsically involved in the regulation of inflammatory cytokine genes [50, 51, 52]. NF-κB was evaluated in three (17.6%) of the studies analyzed in this review [27, 29, 35]. In Williams et al. [27], a reduction in nuclear binding activity NF-κB and NF-IL6 in addition to

TNF-α and IL-6 levels was observed regardless of glucan treatment schedule (pre- and postsepsis treatment). In the studies by Ha et al. [29] and Liu et al. [35], it was demonstrated that glucans increase NF-κB activation. Also, Liu et al. [35] showed decreased TNF-α and IL-6 quantities following glucan treatment. These variable results could be related to the different experimental approaches adopted in each study.

The expression of TLR-2 and TLR-4 in patients with sepsis was upregulated compared to that of healthy individuals [53, 54]. In this review, two studies (11.7%) evaluated these receptors; both reported a decrease in the expression of TLR-2 and TLR-4 [29, 30]. In these studies, the treatment protocol, form of administration, and evaluation period were identical, differing only in the dose administered, specifically 40 mg/kg for Ha et al. [29] and 50 mg/kg for Williams et al. [30].

The mechanisms underlying the protective effects of glucans against CLP–induced sepsis are due to an enhanced host immune response against the infection. A key aspect is the modulation of the expression of mortality-related genes, such as the overexpression of TLRs, contributing to the progression of the inflammatory injury cycle during sepsis [17]. Thus, treatment with glucans, by modulating the expression of these genes, attenuated proinflammatory responses through signal transduction pathways mediated by downregulating receptors, which may lead to improved long-term survival. Additionally, glucans activate the phosphoinositide 3-kinase signaling pathway in CLP sepsis; this pathway limits the activation of signaling and the expression of proinflammatory mediators [55, 56].

Another important assay when studying septic conditions is the level of myeloperoxidase (MPO), as it is directly related to neutrophil infiltration and activation. In Babayigit [19], Bedirli [20], and Demir et al. [38], the production of this enzyme was evaluated, and its increase in CLP animals was attenuated by β-glucan treatment [19, 20, 38]. Also, MDA, a biomarker of lipid peroxidation induced by oxidative stress and indicative of severe sepsis [57], was described in three studies [25, 37, 38] corresponding to 17.6% of the total; these demonstrated MDA formation is reduced after treatment with glucans.

It is worth mentioning that different routes of administration were observed among the articles selected (intragastric gavage, intraperitoneal, subcutaneous, intramuscular, and intravenous), with the intraperitoneal route being the most used among the articles included [11]. There is no established consensus on the best route of administration of β-glucan in septic models. Among the articles selected in this study, the intraperitoneal administration achieved greater animals survival and significant immunomodulatory activity. However, it is worth noting that the subcutaneous route was also one of the main choices for the treatment of septic mice [21, 58, 59, 60], reaching satisfactory beneficial results. Thinking ahead and acceptance in humans, the subcutaneous routes have advantages in that no professional qualification is required for application in contrast to IV and IM administrations, the injections are less painful, the risk of infection is lower with SC injections than with IV injections, and, if it does occur, the infeccion is generally limited to a local infection rather than a systemic infection. In addition, SC injections offer a wider range of alternatives sites than IM injections for patients requiring multiple doses [61, 62]. Another systematic study highlighted patient compliance, with time savings and autonomy for home treatment being some of the reasons for preferring the subcutaneous route of administration [63].

The ability of glucans to act as modulators of the inflammatory response not only reveals their therapeutic potential but also highlights their biotechnological relevance, providing prospects for the medical community in the prognosis of septic individuals. In this context, glucans emerge as a promising alternatives as therapies and adjuvants. In a phase 2 randomized clinical trial evaluating the effect of oral β-glucan supplementation, this potential was observed through an increase in IgG in patients who received a vaccine booster associated with β-glucan supplementation. The research aimed to improve IgG antibody titers and seroconversion rates, which are associated with improved survival in patients with high-risk neuroblastoma. To do this, the authors selected 107 patients, divided into groups: group 1 did not receive β-glucan for the first 5 weeks, and group 2 received an oral regimen of β-glucan (40 mg/kg/d for 14 consecutive days and then 14 days without) from week 1. From week 6, all 107 patients received the β-glucan regimen for 1 year or until disease progression. Seven subcutaneous vaccine injections were administered (weeks 1, 2, 3, 8, 20, 32, and 52), each consisting of 30 μg of GD2 and 30 μg of GD3. It was observed that by administering β-glucan early during the initiation phase (group 2), the IgG1 anti-GD2 antibody response in vaccinated patients was statistically higher than in the control (group 1). In addition, a high antibody response ($\geq$230 ng/mL) at week 8 of the vaccine correlated significantly with improved progression-free survival. The authors also emphasized that treatment with β-glucan meets many of the requirements for an effective adjuvant [64]. Vetvicka [65] states that β-glucans have been widely used to protect against infections. Using various experimental models, β-glucans have been shown to protect against bacterial and protozoan infections, as well as to increase the efficacy of antibiotics in infections caused by antimicrobial-resistant bacteria. In addition, this review has shown its effectiveness in modulating immune response mediators such as cytokines, reducing inflammatory infiltrates and preventing tissue damage, corroborating its crucial role in attenuating the exacerbated inflammatory response characteristic of septic conditions. It is necessary to invest in more *in vivo* studies, especially in a mouse model of lethal sepsis, so that the prospects for the future in the clinics can be confirmed, ensuring the use of glucans in systemic infections.

Long-term adverse effects have been debated to ensure the safety of glucan supplementation. Studies such as that by Cardenas et al. [66] evaluate the toxicity of β-glucan extract derived from yeast which was evaluated in 44 patients, and in general, good tolerance was obtained, even at the highest doses. In addition, the 27 patients who survived had no adverse effects after 5 years of treatment. Meng [67] evaluated the ability of β-glucan (orally 1 time/day for 12 weeks) to prevent the number of infections in children during the cold season, and even at higher concentrations, no relevant adverse effects were recorded. In the work of Urbancikova et al. [68], the effect of

β-glucan in the treatment of herpes was observed, and even with the glucan extract being used for 120 consecutive days, no adverse effects were observed. Therefore, the evidence suggests that the use of extracts containing β-glucans has not caused any adverse effects.

Sepsis is a very complex disease which depends on inflammatory and anti-inflammatory pathways to comprise effective immune responses as well as accomplish homeostasis. The intricate network of players and pathways underlying sepsis fatality or recovery means that disease management depends on a timely and appropriate management, which therefore reflect on its outcome. It is important to highlight that sepsis control goes beyond a single compound given at a unique time point, such as the importance of rehydration, hemodynamic support, antibiotic administration, and control of the source of infection, among other measures [69]. This is specially important when nutritional ingredients such as glucans can be taken daily way before and even during the course of sepsis, greatly influencing disease evolution. This means that a successful treatment all depends on a series of factors which include therapy and patients immune background. Nonetheless, glucans as other natural products are powerful resources to be individually considered to sepsis and other mechanistically similar diseases.

Considering the complexity of sepsis, as well as the continuous search for novel effective therapies, glucans have emerged as promising threrapies or adjuvants for conventional treatments. The potential of glucans is evidenced by their ability to modulate the immune response and reduce tissue damage. Glucans therefore appear to be a promising biotechnological alternative for sepsis management.

Data Availability

The article data supporting this review are from previously reported studies and datasets, which have been cited. The processed data are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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References

[1] K. Honeyford, A.-P. Nwosu, R. Lazzarino et al., "Prevalence of electronic screening for sepsis in National Health Service acute hospitals in England," *BMJ Health & Care Informatics Online*, vol. 30, no. 1, Article ID e100743, 2023.

[2] K. E. Rudd, S. C. Johnson, K. M. Agesa et al., "Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study," *The Lancet*, vol. 395, no. 10219, pp. 200–211, 2020.

[3] M. Cecconi, L. Evans, M. Levy, and A. Rhodes, "Sepsis and septic shock," *The Lancet*, vol. 392, no. 10141, pp. 75–87, 2018.

[4] Z. Xu, A. Liu, L. Yang et al., "Changes in immune function and immunomodulatory treatments of septic patients," *Clinical Immunology*, vol. 239, Article ID 109040, 2022.

[5] M. A. McBride, A. M. Owen, C. L. Stothers et al., "The metabolic basis of immune dysfunction following sepsis and trauma," *Frontiers in Immunology*, vol. 11, Article ID 1043, 2020.

[6] Y. Pan, J. Li, X. Xia et al., "β-glucan-coupled superparamagnetic iron oxide nanoparticles induce trained immunity to protect mice against sepsis," *Theranostics*, vol. 12, no. 2, pp. 675–688, 2022.

[7] A. Geller, R. Shrestha, and J. Yan, "Yeast-derived β-glucan in cancer: novel uses of a traditional therapeutic," *International Journal of Molecular Sciences*, vol. 20, no. 15, Article ID 3618, 2019.

[8] S. Aoe, "Beta-glucan in foods and health benefits," *Nutrients*, vol. 14, no. 1, Article ID 96, 2022.

[9] H. S. Goodridge, A. J. Wolf, and D. M. Underhill, "β-glucan recognition by the innate immune system," *Immunological Reviews*, vol. 230, no. 1, pp. 38–50, 2009.

[10] S. J. C. F. M. Moorlag, N. Khan, B. Novakovic et al., "β-glucan induces protective trained immunity against *Mycobacterium tuberculosis* infection: a key role for IL-1," *Cell Reports*, vol. 31, no. 7, Article ID 107634, 2020.

[11] F. Bloos, J. Held, S. Kluge et al., "(1→ 3)-β-D-glucan-guided antifungal therapy in adults with sepsis: the CandiSep randomized clinical trial," *Intensive Care Medicine*, vol. 48, pp. 865–875, 2022.

[12] B. Du, M. Meenu, H. Liu, and B. Xu, "A concise review on the molecular structure and function relationship of β-glucan," *International Journal of Molecular Sciences*, vol. 20, no. 16, Article ID 4032, 2019.

[13] F. Zhu, B. Du, Z. Bian, and B. Xu, "Beta-glucans from edible and medicinal mushrooms: characteristics, physicochemical and biological activities," *Journal of Food Composition and Analysis*, vol. 41, pp. 165–173, 2015.

[14] L. Vannucci, J. Krizan, P. Sima et al., "Immunostimulatory properties and antitumor activities of glucans (review)," *International Journal of Oncology*, vol. 43, no. 2, pp. 357–364, 2013.

[15] P. Mata-Martínez, M. Bergón-Gutiérrez, and C. del Fresno, "Dectin-1 signaling update: new perspectives for trained immunity," *Frontiers in Immunology*, vol. 13, Article ID 812148, 2022.

[16] G. D. Brown and S. Gordon, "A new receptor for β-glucans," *Nature*, vol. 413, pp. 36-37, 2001.

[17] J A. Willment, A S J. Marshall, D M. Reid et al., "The human β-glucan receptor is widely expressed and functionally equivalent to murine Dectin-1 on primary cells," *European Journal of Immunology*, vol. 35, no. 5, pp. 1539–1547, 2005.

[18] L. Ni, I. Gayet, S. Zurawski et al., "Concomitant activation and antigen uptake via human dectin-1 results in potent antigen-specific $CD8^+$ T cell responses," *The Journal of Immunology*, vol. 185, no. 6, pp. 3504–3513, 2010.

[19] S. LeibundGut-Landmann, F. Osorio, G. D. Brown, and C. Reis e Sousa, "Stimulation of dendritic cells via the dectin-1/Syk pathway allows priming of cytotoxic T-cell responses," *Blood*, vol. 112, no. 13, pp. 4971–4980, 2008.

[20] H. Babayigit, C. Kucuk, E. Sozuer, C. Yazici, K. Kose, and H. Akgun, "Protective effect of β-glucan on lung injury after cecal ligation and puncture in rats," *Intensive Care Medicine*, vol. 31, no. 6, pp. 865–870, 2005.

[21] A. Bedirli, M. Kerem, H. Pasaoglu et al., "Beta-glucan attenuates inflammatory cytokine release and prevents acute lung injury in an experimental model of sepsis," *Shock*, vol. 27, no. 4, pp. 397–401, 2007.

[22] R. Whittemore and K. Knafl, "The integrative review: updated methodology," *Journal of Advanced Nursing*, vol. 52, no. 5, pp. 546–553, 2005.

[23] M. Ouzzani, H. Hammady, Z. Fedorowicz, and A. Elmagarmid, "Rayyan—a web and mobile app for systematic reviews," *Systematic Reviews*, vol. 5, no. 1, 2016.

[24] M. J. Page, J. E. McKenzie, P. M. Bossuyt et al., "The PRISMA 2020 statement: an updated guideline for reporting systematic reviews," *International Journal of Surgery*, vol. 88, Article ID 105906, 2021.

[25] N. Senoglu, M. F. Yuzbasioglu, M. Aral et al., "Protective effects of *N*–acetylcysteine and *β*–glucan pretreatment on oxidative stress in cecal ligation and puncture model of sepsis," *Journal of Investigative Surgery*, vol. 21, no. 5, pp. 237–243, 2008.

[26] J. Issara-Amphorn, S. Surawut, N. Worasilchai et al., "The synergy of endotoxin and (1→3)-*β*-D-glucan, from gut translocation, worsens sepsis severity in a lupus model of Fc gamma receptor IIb-deficient mice," *Journal of Innate Immunity*, vol. 10, no. 3, pp. 189–201, 2018.

[27] D. L. Williams, T. Ha, C. Li, J. H. Kalbfleisch, J. J. Laffan, and D. A. Ferguson, "Inhibiting early activation of tissue nuclear factor-*κ*B and nuclear factor interleukin 6 with (1→3)-*β*-D-glucan increases long-term survival in polymicrobial sepsis," *Surgery*, vol. 126, no. 1, pp. 54–65, 1999.

[28] T. Ha, F. Hua, D. Grant et al., "Glucan phosphate attenuates cardiac dysfunction and inhibits cardiac MIF expression and apoptosis in septic mice," *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 291, no. 4, pp. H1910–H1918, 2006.

[29] T. Ha, Y. Xia, X. Liu et al., "Glucan phosphate attenuates myocardial HMGB1 translocation in severe sepsis through inhibiting NF-*κ*B activation," *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 301, no. 3, pp. H848–H855, 2011.

[30] D. L. Williams, T. Ha, C. Li et al., "Modulation of tissue Toll-like receptor 2 and 4 during the early phases of polymicrobial sepsis correlates with mortality," *Critical Care Medicine*, vol. 31, no. 6, pp. 1808–1818, 2003.

[31] C. T. Newsome, E. Flores, A. Ayala, S. Gregory, and J. S. Reichner, "Improved antimicrobial host defense in mice following poly-(1,6)-*β*-D-glucopyranosyl-(1,3)-*β*-D-glucopyranose glucan treatment by a gender-dependent immune mechanism," *Clinical and Vaccine Immunology*, vol. 18, no. 12, pp. 2043–2049, 2011.

[32] D. L. Williams, C. Li, T. Ha et al., "Modulation of the phosphoinositide 3-kinase pathway alters innate resistance to polymicrobial sepsis," *The Journal of Immunology*, vol. 172, no. 1, pp. 449–456, 2004.

[33] A. Bedirli, S. Gokahmetoglu, O. Sakrak, N. Ersoz, D. Ayangil, and H. Esin, "Prevention of intraperitoneal adhesion formation using beta-glucan after ileocolic anastomosis in a rat bacterial peritonitis model," *The American Journal of Surgery*, vol. 185, no. 4, pp. 339–343, 2003.

[34] G. J. Bowers, M. L. Patchen, T. J. MacVittie, E. F. Hirsch, and M. P. Fink, "Glucan enhances survival in an intraabdominal infection model," *Journal of Surgical Research*, vol. 47, no. 2, pp. 183–188, 1989.

[35] D. Liu, M. You, G.-F. Zhao et al., "The novel *α*-glucan YCP improves the survival rates and symptoms in septic mice by regulating myeloid-derived suppressor cells," *Acta Pharmacologica Sinica*, vol. 38, pp. 1269–1281, 2017.

[36] S. Ozdemir, S. Toplan, M. Ercan, I. Bayrak, B. Uruluer, and O. Sunamak, "The effect of *β*-glucan on trace element levels in intra-abdominal sepsis in rats," *Biological Trace Element Research*, vol. 132, pp. 197–206, 2009.

[37] G. Şener, H. Toklu, F. Ercan, and G. Erkanlı, "Protective effect of *β*-glucan against oxidative organ injury in a rat model of sepsis," *International Immunopharmacology*, vol. 5, no. 9, pp. 1387–1396, 2005.

[38] M. Demir, K. Köse, C. Yazıcı, and E. M. Sözüer, "Does *β*-glucan can have protective role against oxidative stress in experimental sepsis model in rats?" *Ulusal Travma ve Acil Cerrahi Dergisi*, vol. 26, no. 5, pp. 657–662, 2020.

[39] M. Wang, Z. Zhang, X. Dong, and B. Zhu, "Targeting *β*-glucans, vital components of the Pneumocystis cell wall," *Frontiers in Immunology*, vol. 14, Article ID 1094464, 2023.

[40] G. Huang and S. Huang, "The structure–activity relationships of natural glucans," *Phytotherapy Research*, vol. 35, no. 6, pp. 2890–2901, 2021.

[41] S.-C. Chang, R. K. Saldivar, P.-H. Liang, and Y. S. Y. Hsieh, "Structures, biosynthesis, and physiological functions of (1,3;1,4)-*β*-D-glucans," *Cells*, vol. 10, no. 3, Article ID 510, 2021.

[42] J. Wagener, K. Striegler, and N. Wagener, "*α*-and *β*-1, 3-glucan synthesis and remodeling," *The Fungal Cell Wall*, vol. 425, pp. 53–82, 2020.

[43] K. Złotko, A. Wiater, A. Waśko et al., "A report on fungal (1→3)-*α*-D-glucans: properties, functions and application," *Molecules*, vol. 24, no. 21, Article ID 3972, 2019.

[44] D. Dhingra, M. Michael, H. Rajput, and R. T. Patil, "Dietary fibre in foods: a review," *Journal of Food Science and Technology*, vol. 49, pp. 255–266, 2012.

[45] M. P. Arena, P. Russo, V. Capozzi et al., "Combinations of cereal *β*-glucans and probiotics can enhance the anti-inflammatory activity on host cells by a synergistic effect," *Journal of Functional Foods*, vol. 23, pp. 12–23, 2016.

[46] E. Johnson, D. T. Førland, L. Sætre, S. V. Bernardshaw, T. Lyberg, and G. Hetland, "Effect of an extract based on the medicinal mushroom *Agaricus blazei* murill on release of cytokines, chemokines and leukocyte growth factors in human blood *Ex Vivo* and *In Vivo*," *Scandinavian Journal of Immunology*, vol. 69, no. 3, pp. 242–250, 2009.

[47] U. Bacha, M. Nasir, S. Iqbal, and A. A. Anjum, "Nutraceutical, anti-inflammatory, and immune modulatory effects of *β*-glucan isolated from *Yeast*," *BioMed Research International*, vol. 2017, Article ID 8972678, 14 pages, 2017.

[48] E. Żyła, K. Dziendzikowska, D. Kamola et al., "Anti-inflammatory activity of oat beta-glucans in a Crohn's disease model: time- and molar mass-dependent effects," *International Journal of Molecular Sciences*, vol. 22, no. 9, Article ID 4485, 2021.

[49] E. J. Murphy, C. Masterson, E. Rezoagli et al., "Immunomodulation properties of a novel *β*-glucan extract from the mushroom lentinus edodes in an in-vitro lung injury model," in *A60. LUNG INJURY, SEPSIS, AND ARDS*, pp. A2114–A2114, American Thoracic Society, 2019.

[50] A. S. Baldwin Jr., "The NF-*κ*B and I*κ*B proteins: new discoveries and insights," *Annual Review of Immunology*, vol. 14, pp. 649–681, 1996.

[51] C. Xia, J. K. Cheshire, H. Patel, and P. Woo, "Cross-talk between transcription factors NF-κB and C/EBP in the transcriptional regulation of genes," *The International Journal of Biochemistry & Cell Biology*, vol. 29, no. 12, pp. 1525–1539, 1997.

[52] P. J. Barnes, "Nuclear factor-κB," *The International Journal of Biochemistry & Cell Biology*, vol. 29, no. 6, pp. 867–870, 1997.

[53] H. Tsujimoto, S. Ono, S. Hiraki et al., "A hemoperfusão com fibras imobilizadas com polimixina B reduziu o número de monócitos CD16+ CD14+ em pacientes com choque séptico," *Journal of Endotoxin Research*, vol. 10, no. 4, pp. 229–237, 2004.

[54] L. Armstrong, A. R. L. Medford, K. J. Hunter, K. M. Uppington, and A. B. Millar, "Differential expression of Toll-like receptor (TLR)-2 and TLR-4 on monocytes in human sepsis," *Clinical and Experimental Immunology*, vol. 136, no. 2, pp. 312–319, 2004.

[55] M. Guha and N. Mackman, "The phosphatidylinositol 3-Kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells," *Journal of Biological Chemistry*, vol. 277, no. 35, pp. 32124–32132, 2002.

[56] D. L. Williams, T. Ozment-Skelton, and C. Li, "Modulation of the phosphoinositide 3-kinase signaling pathway alters host response to sepsis, inflammation, and ischemia/reperfusion injury," *Shock*, vol. 25, no. 5, pp. 432–439, 2006.

[57] S. L. Weiss and C. S. Deutschman, "Elevated malondialdehyde levels in sepsis—something to 'stress' about?" *Critical Care*, vol. 18, Article ID 125, 2014.

[58] A. Khosrojerdi, S. Soudi, A. Zavaran Hosseini, S. Ghaffari Khaligh, and S. M. Hashemi, "Imipenem alters systemic and liver inflammatory responses in CLP–induced sepsis mice in a dose-dependent manner," *International Immunopharmacology*, vol. 93, Article ID 107421, 2021.

[59] D. Knappe, R. Schmidt, K. Adermann, and R. Hoffmann, "Continuous subcutaneous delivery of proline-rich antimicrobial peptide Api137 provides superior efficacy to intravenous administration in a mouse infection model," *Frontiers in Microbiology*, vol. 10, Article ID 2283, 2019.

[60] M. C. G. Maciel, E. M. S. Fialho, R. N. M. Guerra, V. M. Borges, F. H. Kwasniewski, and F. R. F. Nascimento, "*Tityus serrulatus* scorpion venom improves survival and lung inflammation in lethal sepsis induced by CLP in mice," *Toxicon*, vol. 89, pp. 1–8, 2014.

[61] I. Usach, R. Martinez, T. Festini, and J.-E. Peris, "Subcutaneous injection of drugs: literature review of factors influencing pain sensation at the injection site," *Advances in Therapy*, vol. 36, pp. 2986–2996, 2019.

[62] M. F. Haller, "Converting intravenous dosing to subcutaneous dosing with recombinant human hyaluronidase," *Pharmaceutical Technology*, vol. 31, no. 10, pp. 118–132, 2007.

[63] K. L. Stoner, H. Harder, L. J. Fallowfield, and V. A. Jenkins, "Intravenous versus subcutaneous drug administration. Which do patients prefer? A systematic review," *The Patient - Patient-Centered Outcomes Research*, vol. 8, pp. 145–153, 2015.

[64] I. Y. Cheung, A. Mauguen, S. Modak et al., "Effect of oral β-glucan on antibody response to ganglioside vaccine in patients with high-risk neuroblastoma: a phase 2 randomized clinical trial," *JAMA Oncology*, vol. 9, no. 2, pp. 242–250, 2023.

[65] V. Vetvicka, "Glucan-immunostimulant, adjuvant, potential drug," *World Journal of Clinical Oncology*, vol. 2, no. 2, pp. 115–119, 2011.

[66] F. I. Cardenas, A. Mauguen, I. Y. Cheung et al., "Phase I trial of oral yeast-derived β-glucan to enhance anti-GD2 immunotherapy of resistant high-risk neuroblastoma," *Cancers*, vol. 13, no. 24, Article ID 6265, 2021.

[67] F. Meng, "Baker's yeast beta-glucan decreases episodes of common childhood illness in 1 to 4 year old children during cold season in China," *Journal of Nutrition & Food Sciences*, vol. 6, no. 4, Article ID 518, 2016.

[68] I. Urbancikova, D. Hudackova, J. Majtan, Z. Rennerova, P. Banovcin, and M. Jesenak, "Efficacy of pleuran (β-glucan from *Pleurotus ostreatus*) in the management of herpes simplex virus type 1 infection," *Evidence-Based Complementary and Alternative Medicine*, vol. 2020, Article ID 8562309, 8 pages, 2020.

[69] A. Purcarea and S. Sovaila, "Sepsis, a 2020 review for the internist," *Romanian Journal of Internal Medicine*, vol. 58, no. 3, pp. 129–137, 2020.

[70] M. Burgelman, C. Vandendriessche, and R. E. Vandenbroucke, "Extracellular vesicles: a double-edged sword in sepsis," *Pharmaceuticals*, vol. 14, no. 8, Article ID 829, 2021.

4.2. ARTIGO ORIGINAL

Auricularia auricula's polysaccharides confers protection in murine Sepsis via Immune Modulation

Jesse Pereira Machado Viana[1], Luisa Coutinho Coelho[1], Priscila M. Mendes[3], André Alvares Marques Vale[3], Jeferson Noslen Casarin[2], Joicy Cortez de Sá Sousa[5], Paulo Vitor Soeiro Pereira[3] Anamelia L. Bocca[1,4], Márcia C. G. Maciel[1,2,3]

[1] Departamento de Biologia Celular, Instituto de Ciências Biológicas, Programa de Pós-graduação em Ciências Biológicas (Biologia Molecular), Laboratório de Imunologia Aplicada, Universidade de Brasília (UnB), Brasília, Brazil.

[2] Programa de Pós-graduação em Saúde e Tecnologia, Universidade Federal do Maranhão, Brazil

[3] Programa de Pós-graduação em Ciências da Saúde, Universidade Federal do Maranhão, Brazil

[4] Plataforma Bi-Institucional de Pesquisa Translacional - Fiocruz/SP, Brazil

[5] Universidade Ceuma, Brazil, Programa de pós-graduação em Biosciências Aplicadas à Saúde

Correspondence should be addressed to Márcia C. G. Maciel: marcia.maciel@unb.br

ABSTRACT:

Sepsis is a life-threatening condition characterized by severe organ dysfunction resulting from an uncontrolled host response to infection. The sepsis treatment is a challenge due to its complexity and the need for effective therapeutic approaches. The objective of this study was to evaluate the immunomodulatory and antimicrobial effects of exopolysaccharides (EPS) from *Auricularia auricula* in a murine model of lethal sepsis. Our findings demonstrated that treatment with EPS significantly enhances the proliferation of circulating lymphocytes and granulocytes, which is associated with improved host defense mechanisms. Additionally, EPS administration effectively mitigates sepsis-induced pulmonary damage, as evidenced by preserved lung function. Furthermore, β-glucan treatment significantly reduces microbial translocation to the bloodstream, maintaining a lower colony-forming unit (CFU) count and thereby restricting systemic infection. The polysaccharides also prevent the development of thrombocytopenia in septic animals, thereby preserving

platelet homeostasis. The compound modulates serum cytokine profiles, contributing to a more regulated inflammatory response. The interactome analysis reveals that the Dectin-1→Syk pathway activates NF-κB/AP-1 and involves p38 MAPK, thereby explaining the observed cytokine modulation. The presence of Nod2/Ripk2 suggests that the EPS primes leukocytes to effectively combat both fungi and bacteria, leading to a reduced bacterial load and increased survival. Collectively, these data indicate that EPS, a prebiotic agent, represents a novel therapeutic strategy with potential for modulating immune responses, controlling microbial proliferation, and improving survival outcomes in a model of severe sepsis.

keywords: infection; inflammation; beta-glucan; polysaccharides; prebiotic; survival; cytokines;

1. INTRODUCTION

Sepsis is a potentially fatal organ dysfunction resulting from a dysregulated host response to infection (Singer et al., 2016). This definition underscores the complex and heterogeneous nature of the phenomenon, highlighting that an uncontrolled host response can eventually lead to multiple organ dysfunction syndrome (MODS) due to various complications. Furthermore, an exacerbated inflammatory response can precipitate septic shock. This critical condition arises when a patient becomes unresponsive to fluid resuscitation, developing persistent hypotension accompanied by cellular and metabolic dysfunctions. These dysfunctions significantly increase the risk of mortality in sepsis (Levy et al., 2003).

Sepsis poses a severe public health problem due to its high incidence, mortality rates, and significant treatment costs. Recent epidemiological data from 2020 reveal that sepsis affects approximately 49 million people annually worldwide, resulting in 11 million deaths. This staggering figure represents about 20% of all global deaths (Rudd et al., 2020). The mortality rate from sepsis is particularly high in low and middle-income countries, where limited resources and inadequate medical infrastructure exacerbate the issue. Moreover, it's estimated that one in three cases of sepsis annually results in death (Evans et al., 2021).

A variety of pathogens, including bacteria, viruses, fungi, and protozoa, can cause sepsis in humans, with bacteria being the predominant culprits (Singer et al., 2016). Among the primary bacterial agents, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pneumoniae* are particularly notable, in addition to multidrug-resistant pathogens (Parrillo & dellinger, 2007; Vincent et al., 2020). The increasing antimicrobial resistance further complicates the treatment of sepsis. When the causative pathogens are resistant to multiple medications, therapeutic options become limited, leading to less effective treatments, more prolonged and severe infections, and ultimately, higher mortality rates. Moreover, the direct and indirect costs associated with sepsis treatment impose a significant burden on healthcare systems. This includes extended intensive care, often requiring ventilatory support and surgical interventions, leading to prolonged stays in Intensive Care Units (ICUs) (Paoli et al., 2018). Beyond acute care, there are also substantial costs related to rehabilitation and long-term care, as patients frequently suffer from lasting physical, cognitive, and emotional sequelae (Fleischmann et al., 2016; Iwashyna et al., 2010). Thus, the combination of high mortality, growing antimicrobial resistance, and the substantial economic burden positions sepsis as a critical global health challenge (La Via et al., 2024). This scenario highlights the urgent need for novel alternative and/or complementary therapies that can mitigate the infectious agent's load on the host, as well as strategies to modulate the immune response, ultimately aiming for a better prognosis.

In this context, β-glucans emerge as a promising subject of study for treating infections, given that they are natural and easily obtainable polysaccharides. Found in the cell walls of fungi, yeasts, cereals, and algae, polysaccharides are well-known for their immunomodulatory properties (Vetvicka et al., 2020), exhibiting prebiotic activity that promotes the growth of beneficial bacteria in the gut microbiota while inhibiting the proliferation of undesirable pathogens. Thus, β-glucans play a crucial role in maintaining the balance of the gastrointestinal microbiota (Ciecierska et al., 2019). A systematic review of randomized clinical trials investigates the effect of exclusive oral administration of fungal beta-(1→3,1→6)-D-glucans, in various forms and dosages, on maintaining homeostasis in healthy or susceptible individuals showing that the majority of studies highlighted beneficial effects on

immunomodulation and immune system potentiation. A reduction in the incidence and symptoms of common colds, influenza, and upper respiratory tract infections is evidence of these effects (Vlassopoulou et al., 2021). Further supporting these findings. Walachowski et al (Walachowski et al., 2022) evaluated the effects of oral supplementation with purified β-glucans from *Saccharomyces cerevisiae* in mice infected intraperitoneally (I.P.) with E. coli. The results showed improved infection control, demonstrated by a lower bacterial load and a reduction in tissue damage associated with necrosis in the liver and spleen. Another notable effect of yeast β-glucan treatment is its ability to stimulate the activation of monocytes/macrophages, promoting increased cytokine secretion during a secondary challenge—a phenomenon termed "immune training.". Saeed et al (Saeed et al., 2014) demonstrated *in vitro* that monocytes incubated with β-glucan induce trained immune cells, characterized by an increase in TNF-α and IL-6 secretion. Following an infectious challenge, monocytes can retain this "trained immunity" from a previous encounter, entering a state of either tolerance or heightened responsiveness.

Given the increasing relevance of polysaccharides as therapeutic agents in modulating the immune response, this study aims to investigate the effect of a mushroom-derived polysaccharide, containing a high concentration of β-glucan, in a lethal cecal ligation and puncture (CLP) model of sepsis. Through this investigation, we seek to provide insights into how β-glucans can contribute to mitigating the dysregulated inflammatory response, reducing mortality, and improving clinical outcomes in animals with induced systemic infection. Ultimately, we propose incorporating this polysaccharide into the diets of patients at risk of developing sepsis, suggesting a novel approach to mitigate this life-threatening condition.

2. MATERIALS AND METHODS

2.1. Cultivation of Auricularia auricula and polysaccharide extraction

The *Auricularia auricula* (CC 309) mushrooms were sourced from the Mushroom Germplasm Bank for Human Use at the Brazilian Agricultural Research Corporation (EMBRAPA), Genetic Resources and Biotechnology Unit - Cenargen, under the coordination of Dr. Arailde Fontes Urben. Access to this genetic heritage was

authorized by the National Council for Scientific and Technological Development (CNPq), as documented in authorization term number 010342/2014-1.

The basidiomycete Auricularia auricula was cultivated on solid medium, Potato Dextrose Agar (Acumedia, ref. 7585A), prepared according to the manufacturer's instructions with 10% agar (Kasvi, ref. K25-611001), and incubated at 25°C until the mycelium covered the entire plate area. A submerged culture method was employed to obtain polysaccharides. An initial inoculum was prepared with mycelium fragments in an Erlenmeyer flask containing 100 mL of Potato Dextrose medium. The culture was kept for 7 days at 30°C with agitation. After this period, fresh culture medium was added to the inoculum, and again, it was cultivated under the same conditions. The mycelium was separated by filtration and washed to remove low-molecular-weight components. Subsequently, polysaccharides were extracted as described before (Basso et al., 2020), and were precipitated, centrifuged, and washed with acetone. After washing, the material was resuspended in deionized water and lyophilized before proceeding to further characterization and biological activity assays. The exopolysaccharide composition was described previously (Coelho et al., 2025).

2.2. Murine model of Sepsis

This study utilized female C57BL/6 mice, aged 8–12 weeks (average weight: 25 g). The animals were obtained from the Bioterium of the Federal University of Uberlândia (UFU) and housed in the Bioterium of the Department of Genetics and Morphology (GEM) at the Institute of Biological Sciences (ICB), University of Brasília (UnB). The mice were housed under controlled conditions with a temperature of 26 ± 2°C, relative humidity of 40%–60%, and a 12-hour light/dark cycle, and had free access to food and water. The study was conducted in accordance with the guidelines approved by the Animal Use Ethics Committee of the Federal University of Brasília (Protocol: 23115.002346/2005-45), which considered protocols to minimize pain, suffering, and distress.

Each group used five C57BL/6 mice, in two independent experiments. They were randomly assigned to one of three experimental groups: Sham group, which underwent a laparotomy procedure without cecal ligation and puncture. CLP group:

which was subjected to cecal ligation and puncture (CLP) without any additional treatment. CLP/GLU group: which received prophylactic treatment with EPS (5 mg/kg) via gavage for 15 days before the CLP procedure.

Polymicrobial sepsis was induced using the cecal ligation and puncture method described by Benjamim et al (Benjamim et al., 2000), with minor modifications. Briefly, all mice were intraperitoneally anesthetized with a mixture of 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride. Laparotomy was performed, and the cecum was mobilized, ligated below the cecal valve, and punctured 8 times using an 18-gauge needle to induce lethal sepsis. The cecum was placed back into the peritoneal cavity, and the abdomen was closed in two layers. Saline (0.5 mL/10 g body weight) was administered subcutaneously to the animals as a hydration fluid.

We conducted two distinct in vivo experiments: a survival analysis and an evaluation of cellular, immunological, and functional parameters. In the survival analysis, mice were observed every 24 hours for a period of five days. At the end of the observation period, or if humane endpoints were met, they were euthanized with an anesthetic overdose (150 mg/kg ketamine hydrochloride and 120 mg/kg xylazine hydrochloride) (Oliveira et al., 2019). For the assessment of cellular, immunological, and functional parameters, biological samples were collected 12 hours after the CLP procedure. Blood and tissue samples were then analyzed to evaluate antimicrobial, anti-inflammatory, and immunological (Sousa et al., 2019).

2.3. Evaluation of cellular, immunological, and functional parameters

A survival analysis and an evaluation of cellular, immunological, and functional parameters were conducted. In the survival analysis, mice were observed every 24 hours for a period of five days. At the end of the observation period, or if humane endpoints were met, they were euthanized with an anesthetic overdose (150 mg/kg ketamine hydrochloride and 120 mg/kg xylazine hydrochloride) (Oliveira et al., 2019). For the assessment of cellular, immunological, and functional parameters, biological samples were collected 12 hours after the CLP procedure. Blood and tissue samples were then analyzed to evaluate antimicrobial, anti-inflammatory, and immunological (Sousa et al., 2019).

After euthanasia, blood samples were collected from the retro-orbital plexus into conical plastic tubes pre-coated with EDTA. A Hematoclin Haematology Analyser 2.8 VET - R666 was used to analyze the hematological parameters. These parameters included the percentages of leukocyte subpopulations and their total counts, as well as platelet counts (PLT).

2.4. Determination of Bacterial Burden

To quantify bacterial load, 10 µL samples of both blood and peritoneal lavage fluid were collected. The blood sample was serially diluted (10^2 CFU/mL) by adding 90 µL of phosphate-buffered saline (PBS) to each well of a 96-well plate. The peritoneal lavage fluid sample followed the same dilution parameters, with a serial dilution of 10^5 CFU/mL. Subsequently, 100 µL aliquots of these dilutions were then plated onto Brain Heart Infusion (BHI) agar plates (Difco Laboratories, Detroit) and spread using a Drigalski spatula. The CFUs were counted after overnight incubation at 37°C. Results are expressed as CFU/mL (Sousa et al., 2019).

2.5. Determination of Cellularity

Following euthanasia, the femur, spleen, and mesenteric lymph node were collected. The femur was perfused with 1 mL of ice-cold PBS to isolate bone marrow cells. The spleen was then mechanically dissociated and washed with 5 mL of PBS and subsequently passed through a 70 µm cell strainer. Similarly, the mesenteric lymph node was dissociated and washed with 1 mL of PBS, and then passed through a 70 µm cell strainer. Finally, the isolated cells were stained with 0.05% crystal violet (in 30% acetic acid) and counted using a Neubauer chamber (Sigma–Aldrich) under a light microscope at 400× magnification.

Peritoneal cells were collected by lavaging the peritoneal cavity with 5 mL of sterile, ice-cold PBS. Following collection, the cells were stained with 0.05% crystal violet (in 30% acetic acid) and counted using a Neubauer chamber (Sigma–Aldrich) under a light microscope at 400× magnification.

2.6. Cytokine Quantification

Serum cytokine levels were measured using the BD™ Cytometric Bead Array Mouse Inflammatory Cytokine Kit (Cat. No. 552364). This assay quantified the

concentrations of tumor necrosis factor-alpha (TNF)-α, monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-6, IL-10, IL-12, and interferon (IFN)-γ in serum samples. The procedure followed the protocol described by Hodge et al. (Hodge et al., 2004). Samples were analyzed on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA), and data were processed using FCAP Array Version 3.0 software (BD Biosciences, San Jose, CA, USA). Results are expressed in pg/mL for each cytokine.

2.7. Histological Analysis

Lungs were removed and fixed in 10% formalin. Tissue samples were then processed through a series of alcohol baths and embedded in paraffin. Thin sections (5 μm) were prepared using a microtome, mounted on slides, and stained with hematoxylin and eosin (H&E). These sections were examined for circulatory changes, inflammatory infiltrates, necrosis, and edema. Histological images of the lungs were captured using an Aperio Scanner and analyzed with Aperio ImageScope™ Pathology Slide Visualization software. Images were acquired at various magnifications: 1× (2 mm), 2× (1 mm), 4× (500 μm), 5× (400 μm), 10× (200 μm), and 20× (100 μm).

2.8. Protein-Protein Interaction Network Analysis

To map the functional interaction networks among proteins within the Dectin-1 signaling pathway, we utilized STRING v12. This bioinformatics resource integrates data from diverse sources, including public databases and high-throughput datasets, enabling the creation of detailed maps of the protein interactome. Evidence for protein associations within the STRING database originates from independent sources, referred to as "channels." These include genomic context information (neighborhood, gene fusion, gene co-occurrence), co-expression, text mining, biochemical/genetic data ("experiments"), and previously curated pathway and protein complex datasets ("databases") (Szklarczyk et al., 2019). For our analysis, we queried the default settings for functional interaction networks specific to Dectin-1 signaling proteins in the *Mus musculus*. The association sources employed for identifying functional interactions were text mining, experiments, databases, co-expression, neighborhood, and co-occurrence. In the generated network, nodes represent proteins, and edges

denote protein-protein associations. Interactions were mapped with a medium confidence score (0.400).

2.9. Statistical Analysis

Results are expressed as the mean ± standard deviation (SD). The normality of the data was assessed using the D'Agostino–Pearson test. Statistical analysis was performed using ANOVA with Tukey's post hoc test via GraphPad Prism version 8.0.2 software. Differences were considered statistically significant when $p < 0.05$. For survival analysis, Kaplan–Meier curves were employed, and the log-rank test was used to compare the curves.

3. RESULTS

3.1. EFFECT OF EPS TREATMENT ON PERIPHERAL BLOOD CELL RECRUITMENT IN SEPTIC MICE

Treatment with EPS in septic mice (CLP/GLU) significantly increased the absolute number of lymphocytes ($p = 0.0093$) and granulocytes ($p = 0.0017$) in peripheral blood when compared to the CLP group (Figure 2A). Conversely, the CLP group exhibited a reduction in circulating granulocytes ($p = 0.0138$) (Figure 2A). Furthermore, the CLP group also presented with thrombocytopenia ($p = 0.0427$) when compared to the Sham group (Figure 2B).

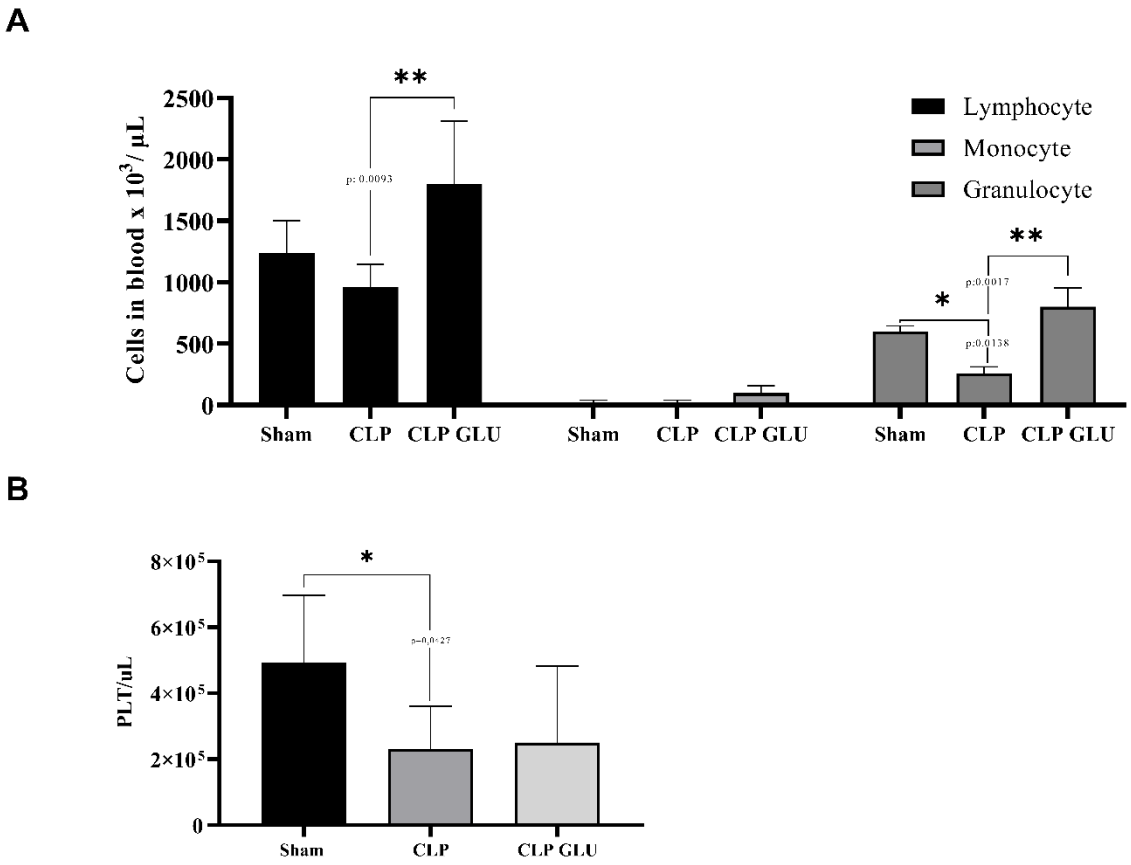

Figure 2. Determination of Absolute Leukocyte and Platelet Counts in Peripheral Blood. The absolute number of leukocytes (A) and platelets (B) in the peripheral blood of the experimental groups. Animals were divided into three groups: Sham: Untreated C57BL/6 mice without sepsis. CLP: Untreated C57BL/6 mice subjected to cecal ligation and puncture (sepsis induction). CLP GLU: C57BL/6 mice were treated via oral gavage with a β-glucan solution (5 mg/kg) for 15 days, and subcutaneously 30 minutes before sepsis induction. Our results show that β-glucan treatment in septic mice led to a significant increase in circulating lymphocytes ($p = 0.0093$) and granulocytes ($p = 0.0138$) compared to the CLP group. No statistical difference was observed between the Sham and CLP GLU groups for these cell types. Furthermore, a reduction in platelet count (thrombocytopenia) was observed between the CLP and Sham groups ($p = 0.0427$). Data are presented as mean ± SD (n=5).

3.2. EFFECT OF EPS TREATMENT ON CELLULARITY OF BONE MARROW, SPLEEN, LYMPH NODE, AND PERITONEUM IN SEPTIC MICE

The CLP group, when compared with the SHAM, showed a reduced cellularity in the bone marrow (Figure 3A), an increase in spleen (Figure 3B), and peritoneum (Figure 3D). The treatment with EPS significantly reduced the number of cells in the bone marrow, compared with SHAM group, and for other analysis there is no difference between these groups. When comparing the CLP-treated and non-treated with EPS, there is a decrease in cell numbers in the spleen and peritoneum. No difference was observed in lymph node cellularity across any groups (Figure 3C).

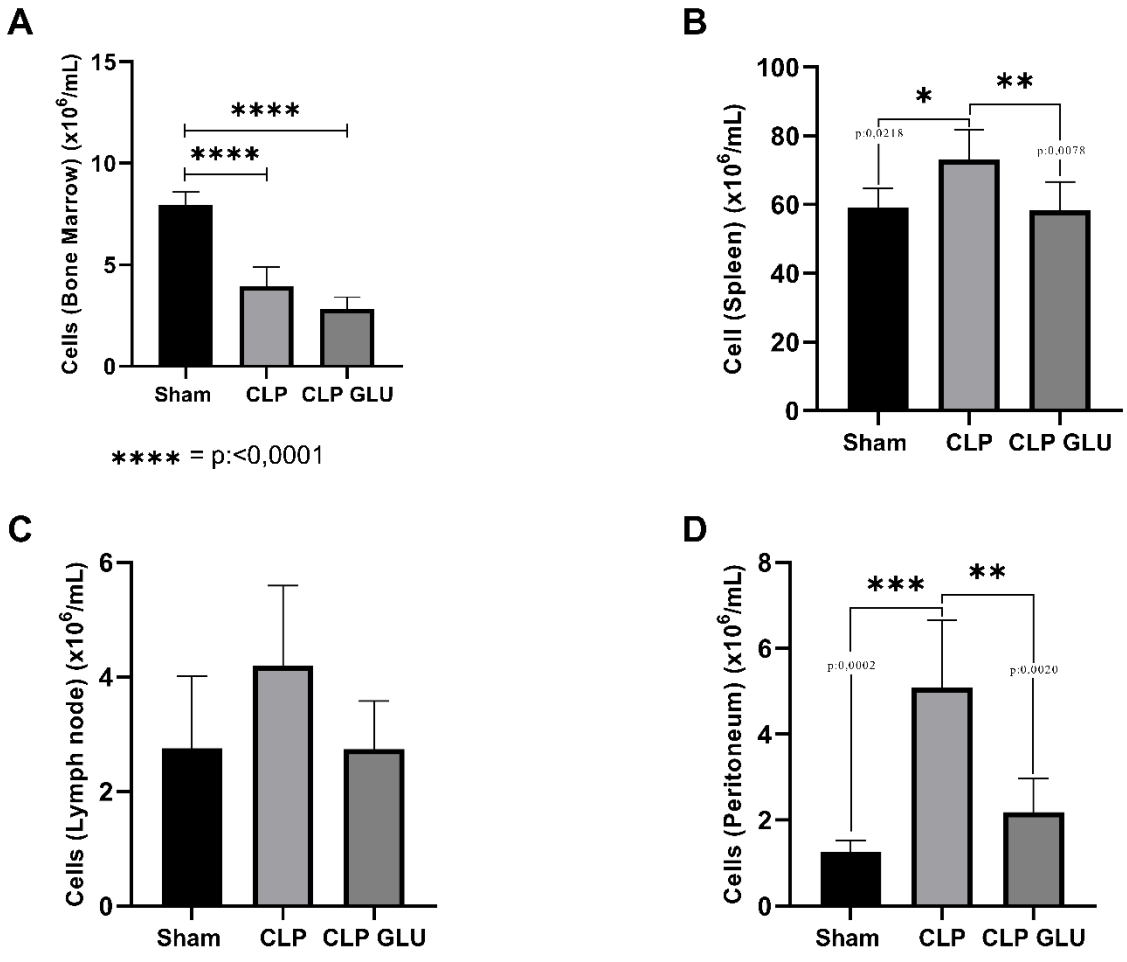

Figure 3. Effect of β-Glucan Treatment on Cellularity of Septic Mice. Animals were divided into three groups: Sham: Untreated C57BL/6 mice without sepsis. CLP: Untreated C57BL/6 mice with sepsis induced by cecal ligation and puncture. CLP GLU: C57BL/6 mice treated via oral gavage with a β-glucan solution (5 mg/kg) for 15 days, and subcutaneously 30 minutes before sepsis induction. Our

findings indicate that β-glucan treatment resulted in a reduction in cell numbers in the bone marrow, spleen, and peritoneum compared to the CLP group. Statistical differences were observed between the CLP and Sham groups for bone marrow, spleen, and peritoneum. No statistically significant difference was found in lymph node cellularity across any of the groups. Data are presented as mean ± SD (n=5).

3.3. EFFECT OF EPS TREATMENT ON LUNG HISTOLOGY IN SEPTIC MICE

Histological analysis was performed to assess hemorrhage in the lungs of septic mice from the Sham, CLP, and CLP GLU groups. The Sham group exhibited normal alveolar architecture, while the CLP group displayed significant alveolar hemorrhage. Notably, the β-glucan-treated group (CLP/GLU) showed a restored normal alveolar structure.

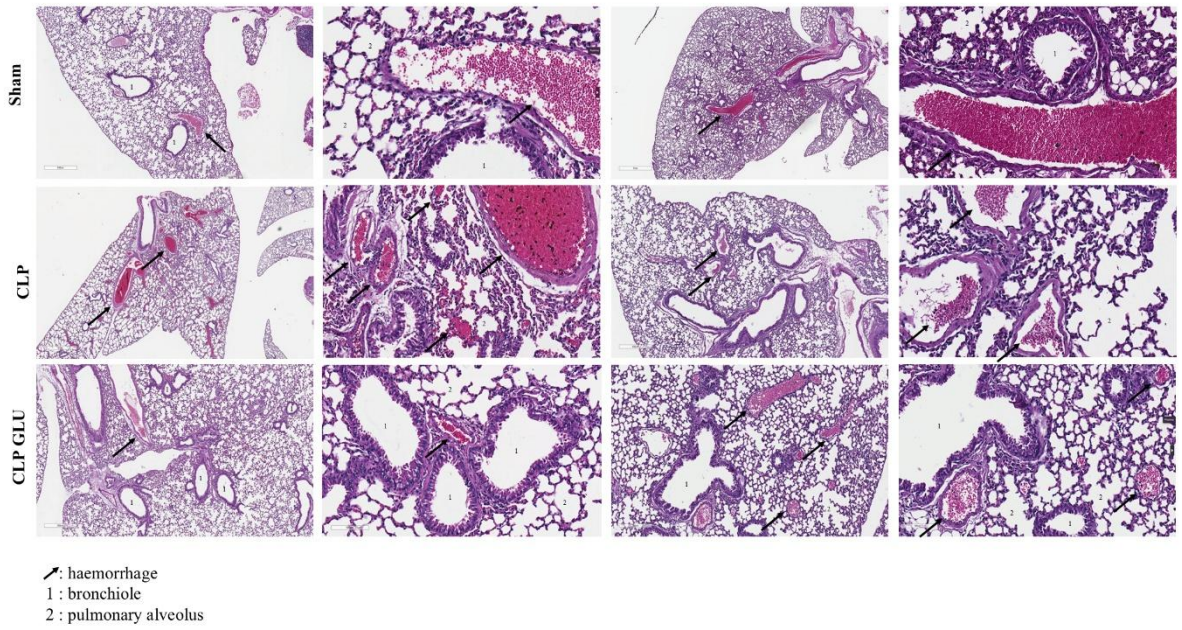

Figure 4. Effect of β-Glucan Treatment on Lung Histology in Septic Mice. Animals were divided into three groups: Sham: Untreated C57BL/6 mice without sepsis. CLP: Untreated C57BL/6 mice with sepsis induced by cecal ligation and puncture. CLP GLU: C57BL/6 mice treated via oral gavage with a β-glucan solution (5 mg/kg) for 15 days, and subcutaneously 30 minutes before sepsis induction. Images were acquired at various magnifications: 1× (2 mm), 2× (1 mm), 4× (500 µm), 5× (400 µm), 10× (200 µm), and 20× (100 µm).

In the Sham group, the lung architecture was preserved, with normal bronchioles and alveolar sacs, and no signs of edema, inflammatory infiltrates, or interalveolar septal thickening. However, a minor circulatory alteration was noted in the

peribronchiolar vessels. In contrast, the CLP group, while generally maintaining preserved pulmonary architecture with normal bronchioles and alveolar sacs, exhibited moderate to severe congestion in the peribronchiolar vessels and a heightened presence of leukocytes adhering to the vascular endothelium, suggesting potential future alveolar infiltration. Importantly, in the CLP GLU group, the lung architecture remained normal, with no significant histological changes, although mild to moderate congestion was observed in the peribronchiolar vessels.

3.4. EFFECT OF EPS TREATMENT ON SERUM CYTOKINE RELEASE IN SEPTIC MICE

The CLP induced an increase in all cytokines when compared with the SHAM group (Figure 5). The analysis of serum cytokine levels revealed that EPS treatment significantly reduced systemic levels of TNF-α ($p<0.0001$), MCP-1 ($p<0.0001$), IL-10 ($p<0.0001$), and IFN-γ ($p = 0.0005$) in septic mice compared to the CLP group (Figure 5). However, this treatment also resulted in a significant increase in IL-6 levels compared to both the CLP group ($p = 0.0030$) and the Sham group ($p<0.0001$). Additionally, IL-12 levels showed a significant difference only between the CLP and Sham groups ($p = 0.0100$).

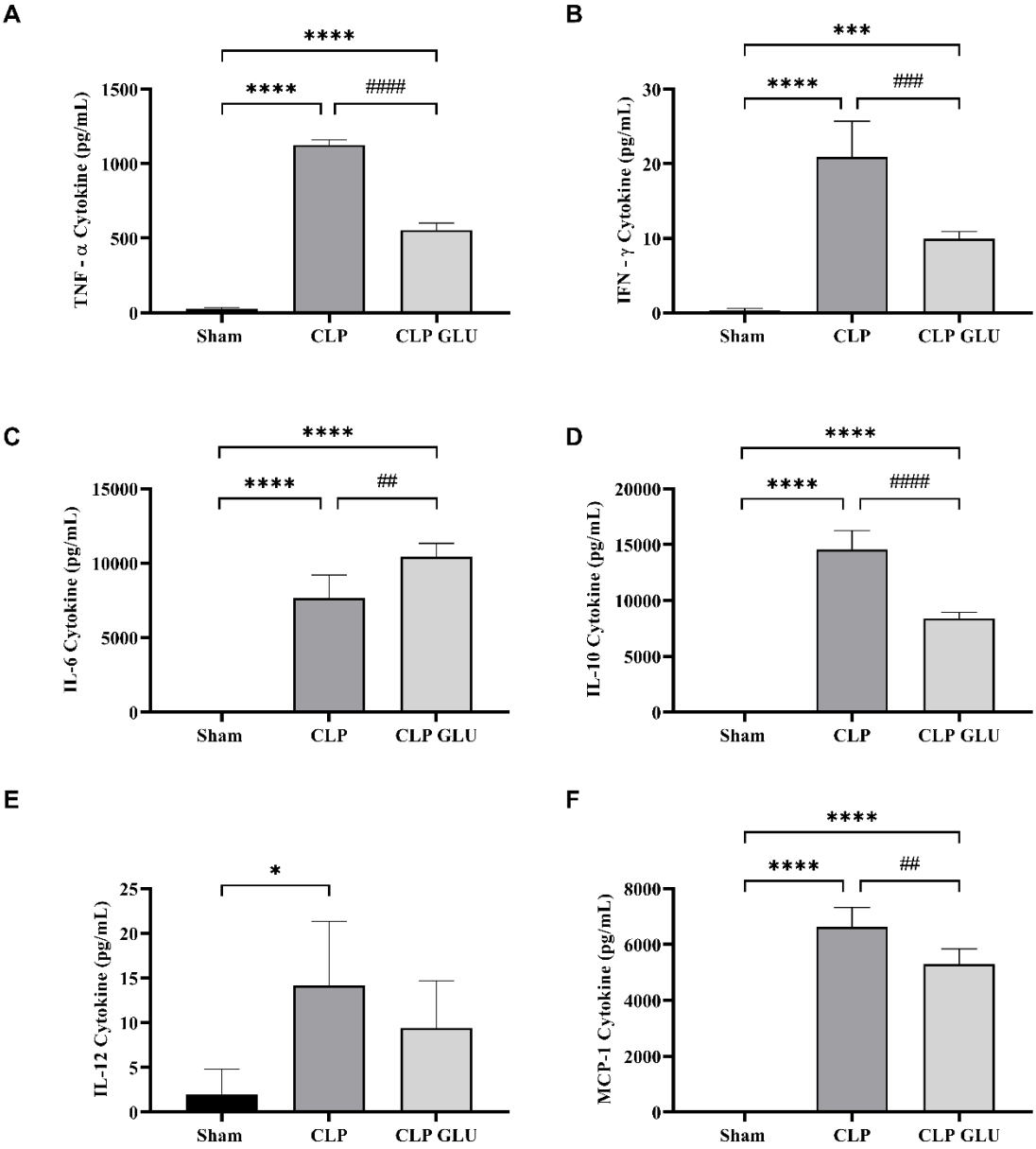

Figure 5: Effect of β-Glucan Treatment on Serum Cytokine Release in Septic Mice. Animals were divided into three groups: Sham: Untreated C57BL/6 mice without sepsis. CLP: Untreated C57BL/6 mice with sepsis induced by cecal ligation and puncture. CLP GLU: C57BL/6 mice were treated via oral gavage with a β-glucan solution (5 mg/kg) for 15 days, and subcutaneously 30 minutes before sepsis induction. After serum collection, levels of IL-6, IL-10, MCP-1, IFN-γ, TNF-α, and IL-12 were evaluated. Data are presented as mean ± SD (n=5).

3.5. ANTIMICROBIAL EFFECT OF EPS TREATMENT IN SEPTIC MICE

In septic mice, β-glucan treatment led to a significant reduction in CFU levels in both the peritoneum (Figure 6A) and peripheral blood (Figure 6B) compared to the CLP group. Our findings demonstrate the inhibition of bacterial translocation into the bloodstream as a result of β-glucan administration.

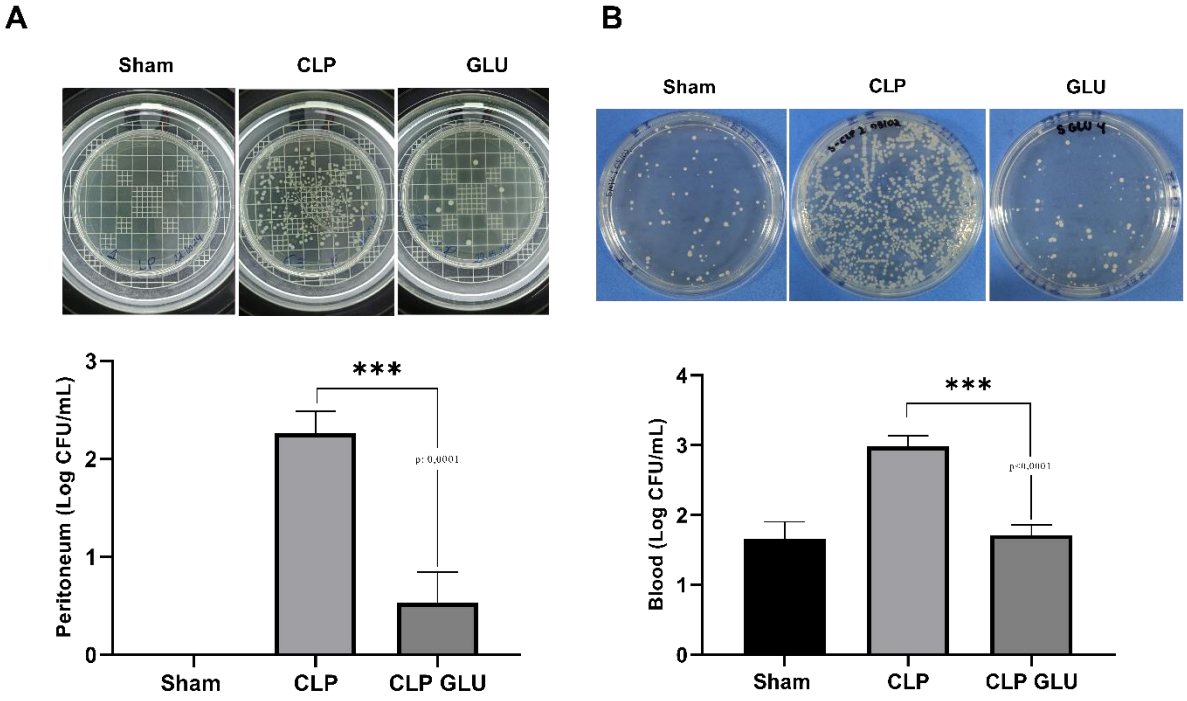

Figure 6. β-Glucan Reduced Colony-Forming Units (CFUs) in the Peritoneum (A) and Peripheral Blood (B). Animals were divided into three groups: Sham: Untreated C57BL/6 mice without sepsis. CLP: Untreated C57BL/6 mice with sepsis induced by cecal ligation and puncture. CLP GLU: C57BL/6 mice treated via oral gavage with a β-glucan solution (5 mg/kg) for 15 days and subcutaneously 30 minutes before sepsis induction. Data are presented as mean ± SD (n=5).

3.6. EFFECT OF EPS TREATMENT ON SURVIVAL OF SEPTIC MICE

All mice in the control CLP group (100%) died within 1 day following the induction of lethal sepsis. In contrast, all Sham group mice (100%) survived. Notably, 60% of the mice in the CLP GLU group survived until the 5th day after sepsis induction (Figure 7).

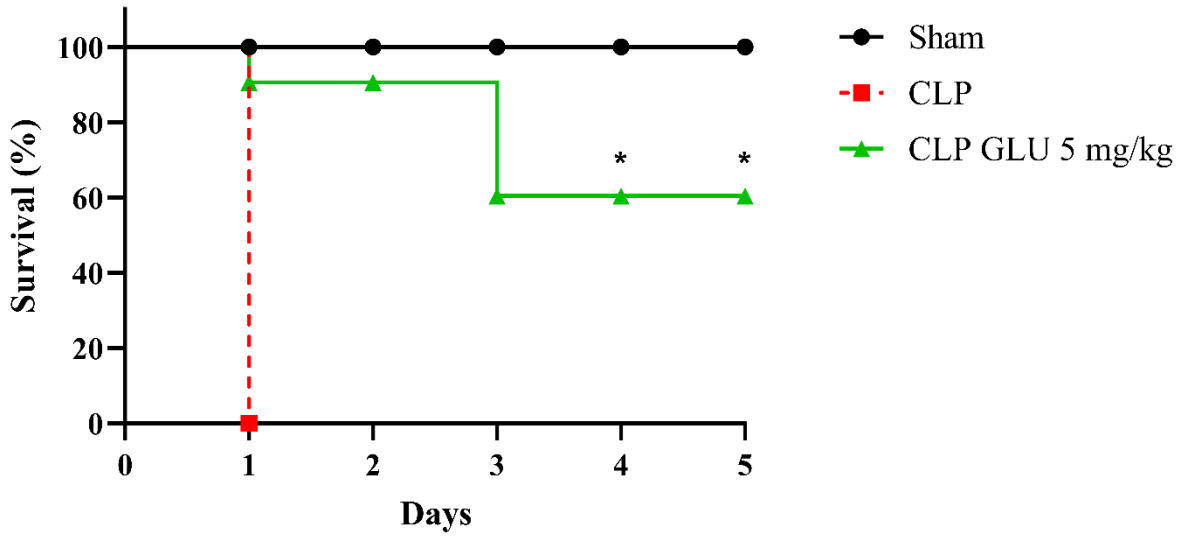

Figure 7: β-Glucan Increased Survival Rate. β-glucan treatment increased survival to 80% by the second day of observation and 60% by the fifth day of observation. Animals were divided into three groups: Sham: Sham-operated mice without sepsis. CLP: Untreated mice with sepsis induced by the CLP model. CLP GLU: Mice treated with β-glucan (5 mg/kg) orally for 15 days and subcutaneously 30 minutes before sepsis induction. Data are presented as mean ± SD (n=5). Asterisks (*) denote a significant difference compared to the CLP group.

3.7. PROTEIN-PROTEIN INTERACTION NETWORK ANALYSIS

In living cells, proteins do not function in isolation; instead, they establish intricate networks of functional interactions that underpin cellular processes. To further elucidate the interaction of Dectin-1 with its associated proteins and their impact on cellular functions, we performed a protein-protein interaction network analysis using the STRING system (https://string-db.org/). The results were used to generate an interactome (Figure 8).

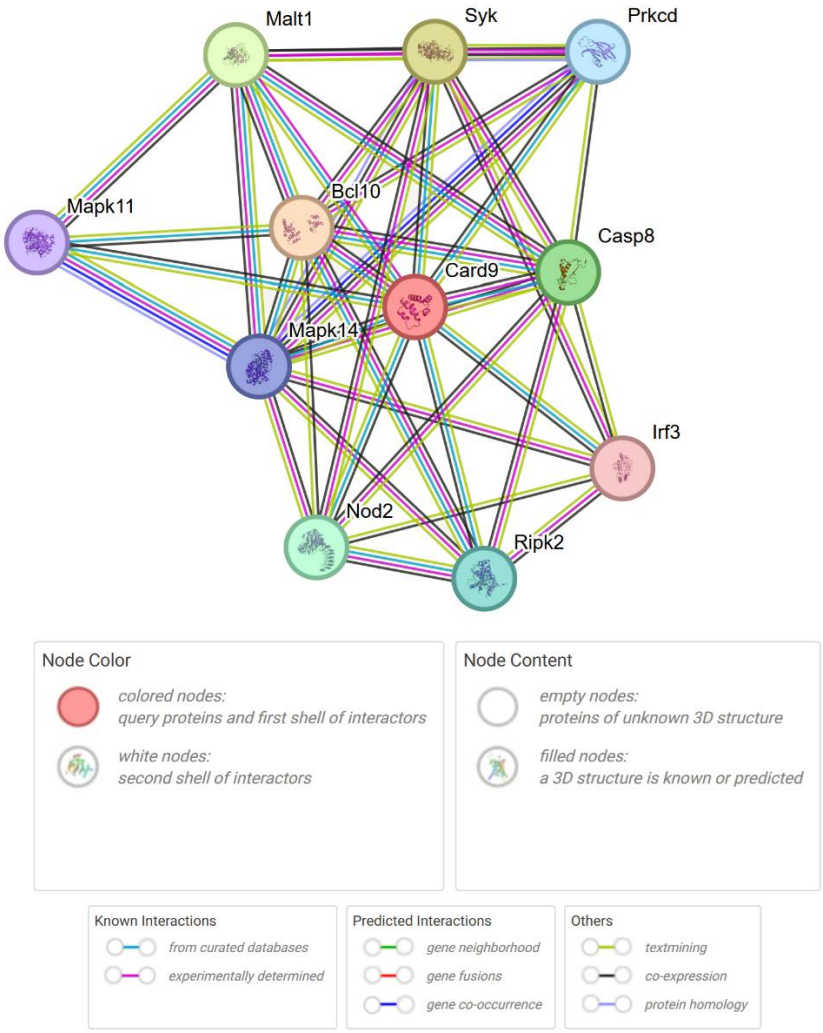

Figure 8: Protein-Protein Interaction Network Analysis using the STRING System. Nodes represent proteins and edges represent protein-protein associations. Interactions were mapped with a confidence score of 0.4.

The STRING analysis revealed a significant protein association network (Figure 8). The central cluster identified included proteins involved in the immune response, specifically in cytokine production (Syk, Card9, Malt1, Bcl10, Casp8, Mapk11, Mapk14, Irf3, Nod2, Ripk2). Furthermore, the analysis also highlighted other biological processes in which these proteins participate, namely the lipopolysaccharide-mediated signaling pathway, response to muramyl dipeptide, and the stress-activated MAPK cascade.

4. DISCUSSION AND CONCLUSION

Sepsis involves a complex immune response, characterized by both pro-inflammatory and immunosuppressive alterations. This includes a significant increase in the production of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, and TNF-α, along with chemokines that help mobilize immune cells to the infection site (Hotchkiss et al., 2013; van der Poll et al., 2017). During this process, which initially presents as an intense inflammatory response, a subsequent phase of immunosuppression can develop. Here, the function of immune cells becomes compromised, predisposing the individual to a numerical loss and/or functional impairment of T cells. This contributes to the worsening clinical outcomes observed in septic patients (Venet & Monneret, 2018).

Numerous studies have consistently shown that lymphocyte counts decrease in the initial phase of sepsis, a phenomenon associated with increased mortality in septic patients. Drewry et al (Drewry et al., 2014) observed that persistent severe lymphopenia (absolute lymphocyte count $\leq 0.6\times10^3$ cells/μL on the fourth day post-sepsis diagnosis) is linked to an elevated risk of mortality. Similarly, Girardot et al (Girardot et al., 2017) identified lymphopenia as a predictor of mortality, demonstrating a higher risk of death in septic shock patients admitted to the ICU. This finding is further corroborated by Vahedi et al (Sheikh Motahar Vahedi et al., 2019), who also highlighted lymphocytopenia as a mortality predictor in septic patients. In another study, Jie Jiang et al (Jiang et al., 2019) demonstrated that lymphocytopenia is prevalent in most severe bacterial infections, even when neutrophil counts are not necessarily elevated. In such cases, patients with neutropenia are even more vulnerable to invasive infections that can rapidly progress to septic shock and death (Clarke et al., 2013). Our results, presented in Figure 3, indicate that β-glucan treatment (CLP GLU) significantly increased the number of circulating lymphocytes, suggesting a potential protective effect. This increase may prevent the lymphopenia frequently associated with high mortality in sepsis. A similar result was observed for granulocyte counts. This rise is likely related to the critical functions granulocytes perform during sepsis, particularly neutrophils, which are rapidly mobilized from the bloodstream to infection sites (Delano & Ward, 2016a, 2016b). The increased absolute number of granulocytes observed in the present study suggests a possible

proliferation of neutrophils, which are key cells in the innate immune response during infectious and inflammatory processes. While activated neutrophils can release neutrophil extracellular traps (NETs), structures crucial for pathogen containment, their unregulated production can contribute to tissue and vascular damage (Brinkmann et al., 2004; Papayannopoulos, 2018). Excessive NET formation can be linked to the development of vasculitis, an inflammatory condition of blood vessels that can lead to vascular compromisse (Kessenbrock et al., 2009). However, in our current study, we observed an absence of histopathological signs of severe inflammation and a preservation of pulmonary architecture, suggesting a potential control over neutrophilic activation. One factor that might have contributed to this outcome is the control of TNF-α. Although a beneficial cytokine, dysregulated TNF-α can lead to pathological conditions. In essence, it is plausible that the modulation of neutrophilic activity mediated by TNF-α prevented the unregulated formation of NETs and, consequently, the development of vasculitis, thereby protecting tissues from collateral damage. This finding aligns with studies highlighting the role of cytokines in regulating NET release and maintaining tissue homeostasis (Kaplan & Radic, 2012; Masuda et al., 2016). Alves-Filho et al (Alves-Filho et al., 2005) also underscored the importance of neutrophils in sepsis infection resolution, correlating the worsening of septic conditions with a failure in neutrophil migration toward the infection site. This mechanism is dependent on the TLR-4 receptor, as it was not observed in TLR-4 knockout mice. Our results provide evidence that β-glucan treatment was capable of preventing this failure in neutrophilic migration to the infectious focus, thereby reducing the vulnerability of the animals.

Beyond lymphocytes and granulocytes, platelets are another crucial component of the immune response significantly affected during sepsis. Thrombocytopenia, or a decrease in platelet count, occurs in septic individuals due to multiple factors including blood dilution, decreased production, excessive consumption, and/or destruction of platelets (Vardon-Bounes, Gratacap, et al., 2019; Vardon-Bounes, Ruiz, et al., 2019). This phenomenon serves as an indicator of sepsis severity due to the critical role platelets play in both the inflammatory response and coagulation during infection. Platelets are not only essential for hemostasis but also actively participate in the immune response by promoting neutrophil recruitment (Grommes

et al., 2012). In the context of sepsis, platelet activation occurs through direct interaction with pathogens, the inflammatory response, and coagulation cascades, all triggered by endothelial tissue damage (Vardon-Bounes, Gratacap, et al., 2019; Vardon-Bounes, Ruiz, et al., 2019). However, excessive activation and consumption of platelets can lead to a significant reduction in their numbers, worsening tissue perfusion, increasing the risk of bleeding, and contributing to organ dysfunction. Consequently, thrombocytopenia becomes a negative prognostic marker in sepsis (Levi & van der Poll, 2017). Our findings indicate that β-glucan treatment did not cause platelet depletion (Figure 2B), suggesting it does not interfere with the mechanisms that ensure vascular integrity and prevent hemorrhage.

Sepsis is known to induce injury and malfunction in multiple organs, with the lungs being among the most severely affected. Sepsis-induced lung injury is strongly associated with morbidity and mortality, primarily driven by the disruption of the endothelial barrier (Dolmatova et al., 2022). Other contributing factors to lung injury are linked to Acute Respiratory Distress Syndrome (ARDS), which is characterized by an exacerbated inflammatory response in the lungs, leading to pulmonary edema and, consequently, respiratory failure (Sheu et al., 2010). In our histological analysis of the lung (Figure 4), we observed no signs indicative of endothelial disruption in the β-glucan-treated animals. Furthermore, the results show that animals treated with β-glucan (CLP GLU) exhibited characteristics similar to those of uninfected animals (Sham): namely, the absence of edema and inflammatory infiltrate, although mild to moderate congestion was noted. This protective effect becomes even more evident when compared to septic animals (CLP), which displayed moderate to severe congestion and potential alveolar infiltration. These findings strongly suggest that the β-glucan treatment preserves normal pulmonary architecture, preventing endothelial barrier rupture and pulmonary edema formation, both of which are factors strongly associated with mortality in sepsis (Dolmatova et al., 2022).

Our analysis of cellularity in various tissues revealed significant alterations in the immune response between the treated (CLP GLU) and untreated (CLP) groups. In the bone marrow, a reduction in cell numbers was observed in both groups, likely

reflecting the mobilization of cells to the infectious focus—a common and expected phenomenon in sepsis (Delano & Ward, 2016b). In the spleen, the increased cellularity in the CLP group could be linked to an exacerbated immune response, characteristic of the hyperinflammation seen in sepsis. Conversely, the reduction in cellularity in the CLP GLU group suggests a potential modulatory effect of β-glucan, controlling cell migration and activation. In the peritoneum, the primary site of infection in the CLP model, the increase in cells in the CLP group reflects the massive recruitment of leukocytes to the site. In contrast, the treated group showed a decrease, suggesting that β-glucan may have reduced excessive cellular recruitment. No statistically significant difference was observed in the cellularity of the lymph nodes among the groups.

Cytokines play a crucial role in the pathology of sepsis, acting as key molecules in the host's inflammatory response to infection. In sepsis, there is an excessive release of pro-inflammatory mediators, which contribute to tissue damage (Dinarello, 2000). However, a phase of immunosuppression also occurs, characterized in part by the presence of anti-inflammatory mediators (Hotchkiss & Nicholson, 2006). Our study found that treatment with β-glucan from Auricularia auricula maintained control over important inflammatory cytokines, specifically reducing TNF-α, IFN-γ, and MCP-1. It also suppressed the anti-inflammatory cytokine IL-10. Furthermore, this treatment stimulated the production of IL-6 and did not interfere with IL-12 levels.

TNF-α is a pivotal cytokine that initiates cascades of other inflammatory cytokine production, playing a crucial role in the onset of the immune response (Tracey et al., 1987). However, its role can be either detrimental or protective depending on the experimental model. Early studies correlated increased TNF-α secretion with elevated mortality when stimulated by LPS injection or *Escherichia coli* (Beutler et al., 1985; Tracey et al., 1987). Other research has yielded similar results (Suffredini et al., 1989; Taveira da Silva et al., 1993; Waage et al., 1987). Our current study demonstrated that β-glucan treatment reduced TNF-α production. This effect likely contributed to the improved survival of the animals and prevented tissue damage that can arise from excessive TNF-α levels.

IFN-γ is a cytokine regulated by the action of IL-12 and IL-18, which promote the activation of macrophage effector functions, leading to pathogen elimination via the production of nitric oxide (NO) and reactive oxygen species (ROS) (Lionakis et al., 2023; Romero et al., 2010). In sepsis, high levels of IFN-γ, as well as its administration after CLP, are related to the intensification of inflammation and consequently increased mortality (Miles et al., 1994). Conversely, the blockade or reduction of IFN-γ is associated with attenuated inflammation and increased survival (Dinges & Schlievert, 2001; Yin et al., 2005; Yin et al., 1999). In the animals treated with β-glucan, a reduction in IFN-γ production was observed.

MCP-1 (monocyte chemoattractant protein-1) is a chemokine frequently associated with the worsening of sepsis and the development of multiple organ dysfunction (Wang et al., 2008) . This deterioration occurs due to the excessive recruitment of immune cells to infection sites and the release of tissue-damaging mediators, such as pulmonary myeloperoxidase (MPO). Ramnath et al (Ramnath et al., 2008) observed a direct relationship between decreased levels of MCP-1 and MPO and improvements in histological and hepatic analyses in CLP and LPS models after treatment with MCP-1 blockers. Our results, consistent with the aforementioned study, indicate a reduction in serum MCP-1 levels, which can be correlated with the histological analysis of the lung (Figure 4). This suggests a possible protective effect against pulmonary damage, thereby corroborating the findings of Ramnath et al. (2008).

Interleukin-6 (IL-6) is a cytokine that can exert either beneficial or detrimental effects on survival during bacterial infections, depending on the study model. It has been observed to be beneficial in some contexts (Dalrymple et al., 1996; van der Poll et al., 1997) while being harmful in others (Riedemann et al., 2003). For instance, Dalrymple et al. (1996) found that IL-6-deficient mice showed increased susceptibility to *Escherichia coli* infection, with higher mortality and elevated CFUs in tissues. Similarly, van der Poll et al. (1997) reported that IL-6 knockout (IL-6 KO-/-) mice exhibited impaired defenses against Streptococcus pneumoniae-induced infection, leading to increased mortality and higher CFUs in the lung. Soltys and Quinn (1999) observed an increase in IL-6 production by lymphocytes from mice

pre-treated with β-glucan after LPS stimulation (Soltys & Quinn, 1999). Their study discussed the role of IL-6 in modulating the apoptosis of these cells. This concept is further supported by Naseem et al (Naseem et al., 2018), who reported a reduction in the percentage of apoptosis in peripheral blood mononuclear cells (PBMCs) following IL-6 stimulation. Our findings, consistent with these earlier studies, indicate an increase in serum IL-6 levels (Figure 3) in β-glucan-treated animals. This increase is likely associated with the prevention of lymphopenia in sepsis, thereby contributing to improved outcomes.

The β-glucan treatment led to a reduction in pro-inflammatory cytokines such as TNF-α and IFN-γ, while notably increasing IL-6 levels. The interactome generated provides a molecular map for this complex modulation. Firstly, Dectin-1 activation by β-glucan initiates a cascade involving Syk→CARD9 → BCL10/MALT1. Secondly, this pathway subsequently activates transcription factors like NF-κB and AP-1, which are critical in controlling the expression of various cytokines. The network analysis also highlights the presence of Mapk11 and Mapk14 (p38 MAPK). The p38 pathway is known for its intricate regulation of TNF-α and IL-6 production. It is plausible that β-glucan, acting through this pathway, promotes a selective modulation, decreasing the production of certain cytokines (TNF-α) while sustaining others (IL-6). This sustained IL-6 production may play a protective role in preventing lymphopenia, a common and detrimental feature of sepsis. Furthermore, the presence of Nod2 and Ripk2 in the interactome is particularly intriguing. NOD2 recognizes muramyl dipeptide, a component of the bacterial cell wall. The interaction between the Dectin-1 and NOD2 pathways suggests that β-glucan effectively "primes" leukocytes to respond more effectively not only to fungi but also to bacteria. This mechanism provides a compelling explanation for the observed reduction in bacterial load (CFUs) in both the peritoneum and blood (Figure 6), representing one of the most significant findings of this study. In summary, the interactome analysis demonstrates that Dectin-1 acts as a central hub, integrating signals from diverse pathogens to mount a more balanced and effective immune response. This integrated response ultimately results in the increased survival observed in the treated animals.

β-glucans are frequently highlighted in the literature for their low toxicity risk (Cardenas et al., 2021; Preece et al., 2021; Tatli Seven et al., 2021) and their antimicrobial properties (Wzorek-Łyczko et al., 2024), which enable pathogen eradication. Our results demonstrated that β-glucan reduced CFUs at the infectious focus within the peritoneal cavity and was capable of reducing bacterial translocation into the bloodstream, thereby preventing dissemination to distant tissues. Harriett et al (Harriett et al., 2022) conducted a similar study, observing that immunization with purified β-glucan preparations from *Saccharomyces cerevisiae* (1 dose of 1-4 mg, 7-14 days before sepsis, via intraperitoneal) induced protection against *Candida albicans, Staphylococcus aureus,* and *Escherichia coli*. This treatment resulted in an 80% survival rate up to the 10th day after sepsis induction. In our current work, we demonstrated an increased survival rate in animals treated with β-glucan, with an 80% survival rate by the second day of observation and 60% from the third to the fifth day of observation. This corroborates the data from Harriett et al. (2022) and confirms that β-glucan treatment modulated immunological parameters as demonstrated, inducing protection against polymicrobial sepsis and providing increased survival.

The results presented in this study demonstrate that the impact of β-glucan administration in a septic model is remarkably diverse. Treatment with β-glucans conferred an increase in survival through several mechanisms. These include an elevated absolute number of leukocytes and granulocytes in peripheral blood, indicating a more robust immune response against the infectious agent. We also observed a significant reduction in colony-forming units (CFUs) in both peripheral blood and the infectious focus, highlighting improved control over bacterial dissemination. Furthermore, the preservation of pulmonary architecture was evident, reflecting a mitigation of inflammatory tissue damage and the prevention of acute lung injury.

Given these findings, our discoveries suggest that administering β-glucans can mitigate critical factors that exacerbate sepsis and elevate mortality risk. Furthermore, pre-administration of β-glucans, prior to sepsis development, may be particularly beneficial. This is because it primes and enhances the immune system, enabling it to respond more efficiently to polymicrobial infection. Thus, the possibility of utilizing β-glucans as therapeutic agents and/or adjuvants in the management of sepsis

represents an innovative perspective, aligning prevention with alternative therapy to improve the clinical outcomes of septic patients.

5. REFERENCES

Alves-Filho, J. C., Benjamim, C., Tavares-Murta, B. M., & Cunha, F. Q. (2005). Failure of neutrophil migration toward infectious focus in severe sepsis: a critical event for the outcome of this syndrome. *Memorias Do Instituto Oswaldo Cruz*, *100 Suppl 1 , 223–226.* . https://doi.org/ https://doi.org/10.1590/s0074-02762005000900038

Basso, A. M. M., De Castro, R. J. A., de Castro, T. B., Guimarães, H. I., Polez, V. L. P., Carbonero, E. R.,…Bocca, A. L. (2020). Immunomodulatory activity of β-glucan-containing exopolysaccharides from Auricularia auricular in phagocytes and mice infected with Cryptococcus neoformans. *Med Mycol*, *58*(2), 227-239. https://doi.org/10.1093/mmy/myz042

Benjamim, C. F., Ferreira, S. H., & Cunha, F. d. Q. (2000). Role of Nitric Oxide in the Failure of Neutrophil Migration in Sepsis. *The Journal of Infectious Diseases*, *182*(1), 214-223. https://doi.org/10.1086/315682

Beutler, B., Milsark, I. W., & Cerami, A. C. (1985). Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science*, *229*(4716), 869-871. https://doi.org/10.1126/science.3895437

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S.,…Zychlinsky, A. (2004). Neutrophil extracellular traps kill bacteria. *Science*, *303*(5663), 1532-1535. https://doi.org/10.1126/science.1092385

Cardenas, F. I., Mauguen, A., Cheung, I. Y., Kramer, K., Kushner, B. H., Ragupathi, G.,…Modak, S. (2021). Phase I Trial of Oral Yeast-Derived β-Glucan to Enhance Anti-GD2 Immunotherapy of Resistant High-Risk Neuroblastoma. *Cancers (Basel)*, *13*(24). https://doi.org/10.3390/cancers13246265

Ciecierska, A., Drywień, M. E., Hamulka, J., & Sadkowski, T. (2019). Nutraceutical functions of beta-glucans in human nutrition. *Rocz Panstw Zakl Hig*, *70*(4), 315-324. https://doi.org/10.32394/rpzh.2019.0082

Clarke, R. T., Jenyon, T., van Hamel Parsons, V., & King, A. J. (2013). Neutropenic sepsis: management and complications. *Clin Med (Lond)*, *13*(2), 185-187. https://doi.org/10.7861/clinmedicine.13-2-185

Coelho, L. C., Favilla, L. D., de Castro, T. B., Leal, M. C. B. D. M., Hoffmann, C., & Bocca, A. L. (2025). Auricularia auricula's Exopolysaccharide Mitigates DSS-Induced Colitis Through Dectin–1-Mediated Immunomodulation and Microbiota Remodeling. *Pharmaceuticals*, *18*(8).

Dalrymple, S. A., Slattery, R., Aud, D. M., Krishna, M., Lucian, L. A., & Murray, R. (1996). Interleukin-6 is required for a protective immune response to systemic Escherichia coli infection. *Infect Immun*, *64*(8), 3231-3235. https://doi.org/10.1128/iai.64.8.3231-3235.1996

Delano, M. J., & Ward, P. A. (2016a). Sepsis-induced immune dysfunction: can immune therapies reduce mortality? *J Clin Invest*, *126*(1), 23-31. https://doi.org/10.1172/jci82224

Delano, M. J., & Ward, P. A. (2016b). The immune system's role in sepsis progression, resolution, and long-term outcome. *Immunol Rev*, *274*(1), 330-353. https://doi.org/10.1111/imr.12499

Dinarello, C. A. (2000). Proinflammatory cytokines. *Chest*, *118*(2), 503-508. https://doi.org/10.1378/chest.118.2.503

Dinges, M. M., & Schlievert, P. M. (2001). Role of T cells and gamma interferon during induction of hypersensitivity to lipopolysaccharide by toxic shock syndrome toxin 1 in mice. *Infect Immun*, *69*(3), 1256-1264. https://doi.org/10.1128/iai.69.3.1256-1264.2001

Dolmatova, E. V., Forrester, S. J., Wang, K., Ou, Z., Williams, H. C., Joseph, G.,…Griendling, K. K. (2022). Endothelial Poldip2 regulates sepsis-induced lung injury via Rho pathway activation. *Cardiovasc Res*, *118*(11), 2506-2518. https://doi.org/10.1093/cvr/cvab295

Drewry, A. M., Samra, N., Skrupky, L. P., Fuller, B. M., Compton, S. M., & Hotchkiss, R. S. (2014). Persistent lymphopenia after diagnosis of sepsis predicts mortality. *Shock*, *42*(5), 383-391. https://doi.org/10.1097/shk.0000000000000234

Evans, L., Rhodes, A., Alhazzani, W., Antonelli, M., Coopersmith, C. M., French, C.,…Levy, M. (2021). Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med*, *47*(11), 1181-1247. https://doi.org/10.1007/s00134-021-06506-y

Fleischmann, C., Scherag, A., Adhikari, N. K., Hartog, C. S., Tsaganos, T., Schlattmann, P.,…Reinhart, K. (2016). Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *Am J Respir Crit Care Med*, *193*(3), 259-272. https://doi.org/10.1164/rccm.201504-0781OC

Girardot, T., Rimmelé, T., Venet, F., & Monneret, G. (2017). Apoptosis-induced lymphopenia in sepsis and other severe injuries. *Apoptosis*, *22*(2), 295-305. https://doi.org/10.1007/s10495-016-1325-3

Grommes, J., Alard, J. E., Drechsler, M., Wantha, S., Mörgelin, M., Kuebler, W. M.,…Soehnlein, O. (2012). Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. *Am J Respir Crit Care Med*, *185*(6), 628-636. https://doi.org/10.1164/rccm.201108-1533OC

Harriett, A. J., Esher Righi, S., Lilly, E. A., Fidel, P., Jr., & Noverr, M. C. (2022). Efficacy of Candida dubliniensis and Fungal β-Glucans in Inducing Trained Innate Immune Protection Against Inducers of Sepsis. *Front Cell Infect Microbiol*, *12*, 898030. https://doi.org/10.3389/fcimb.2022.898030

Hodge, G., Hodge, S., Haslam, R., McPhee, A., Sepulveda, H., Morgan, E.,…Zola, H. (2004). Rapid simultaneous measurement of multiple cytokines using 100 microl sample volumes--association with neonatal sepsis. *Clin Exp Immunol*, *137*(2), 402-407. https://doi.org/10.1111/j.1365-2249.2004.02529.x

Hotchkiss, R. S., Monneret, G., & Payen, D. (2013). Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nature Reviews Immunology*, *13*(12), 862-874. https://doi.org/10.1038/nri3552

Hotchkiss, R. S., & Nicholson, D. W. (2006). Apoptosis and caspases regulate death and inflammation in sepsis. *Nat Rev Immunol*, *6*(11), 813-822. https://doi.org/10.1038/nri1943

Iwashyna, T. J., Ely, E. W., Smith, D. M., & Langa, K. M. (2010). Long-term cognitive impairment and functional disability among survivors of severe sepsis. *Jama*, *304*(16), 1787-1794. https://doi.org/10.1001/jama.2010.1553

Jiang, J., Du, H., Su, Y., Li, X., Zhang, J., Chen, M.,…Niu, B. (2019). Nonviral infection-related lymphocytopenia for the prediction of adult sepsis and its persistence indicates a higher mortality. *Medicine (Baltimore)*, *98*(29), e16535. https://doi.org/10.1097/md.0000000000016535

Kaplan, M. J., & Radic, M. (2012). Neutrophil extracellular traps: double-edged swords of innate immunity. *J Immunol*, *189*(6), 2689-2695. https://doi.org/10.4049/jimmunol.1201719

Kessenbrock, K., Krumbholz, M., Schönermarck, U., Back, W., Gross, W. L., Werb, Z.,…Jenne, D. E. (2009). Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med*, *15*(6), 623-625. https://doi.org/10.1038/nm.1959

La Via, L., Sangiorgio, G., Stefani, S., Marino, A., Nunnari, G., Cocuzza, S.,…Maniaci, A. (2024). The Global Burden of Sepsis and Septic Shock. *Epidemiologia (Basel)*, *5*(3), 456-478. https://doi.org/10.3390/epidemiologia5030032

Levi, M., & van der Poll, T. (2017). Coagulation and sepsis. *Thromb Res*, *149*, 38-44. https://doi.org/10.1016/j.thromres.2016.11.007

Levy, M. M., Fink, M. P., Marshall, J. C., Abraham, E., Angus, D., Cook, D.,…Ramsay, G. (2003). 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med*, *31*(4), 1250-1256. https://doi.org/10.1097/01.ccm.0000050454.01978.3b

Lionakis, M. S., Drummond, R. A., & Hohl, T. M. (2023). Immune responses to human fungal pathogens and therapeutic prospects. *Nat Rev Immunol*, *23*(7), 433-452. https://doi.org/10.1038/s41577-022-00826-w

Masuda, S., Nakazawa, D., Shida, H., Miyoshi, A., Kusunoki, Y., Tomaru, U., & Ishizu, A. (2016). NETosis markers: Quest for specific, objective, and quantitative markers. *Clin Chim Acta*, *459*, 89-93. https://doi.org/10.1016/j.cca.2016.05.029

Miles, R. H., Paxton, T. P., Dries, D. J., & Gamelli, R. L. (1994). Interferon-gamma increases mortality following cecal ligation and puncture. *J Trauma*, *36*(5), 607-611. https://doi.org/10.1097/00005373-199405000-00001

Naseem, S., Manzoor, S., Javed, A., & Abbas, S. (2018). Interleukin-6 Rescues Lymphocyte from Apoptosis and Exhaustion Induced by Chronic Hepatitis C Virus Infection. *Viral Immunol*, *31*(9), 624-631. https://doi.org/10.1089/vim.2018.0045

Oliveira, A. S., Nascimento, J. R., Trovão, L. O., Alves, P. C. S., Maciel, M. C. G., Silva, L. D. M.,…Guerra, R. N. M. (2019). The anti-inflammatory activity of Anacardium occidentale L. increases the lifespan of diabetic mice with lethal sepsis. *J Ethnopharmacol*, *236*, 345-353. https://doi.org/10.1016/j.jep.2019.03.014

Paoli, C. J., Reynolds, M. A., Sinha, M., Gitlin, M., & Crouser, E. (2018). Epidemiology and Costs of Sepsis in the United States-An Analysis Based on Timing of Diagnosis and Severity Level. *Crit Care Med*, *46*(12), 1889-1897. https://doi.org/10.1097/ccm.0000000000003342

Papayannopoulos, V. (2018). Neutrophil extracellular traps in immunity and disease. *Nat Rev Immunol*, *18*(2), 134-147. https://doi.org/10.1038/nri.2017.105

Parrillo, J., & dellinger, R. (2007). Critical Care Medicine: Principles of Diagnosis and Management in the Adult.

Preece, K. E., Glávits, R., Murbach, T. S., Endres, J. R., Hirka, G., Vértesi, A., & Szakonyiné, I. P. (2021). Assessment of toxicological potential of sodium carboxymethyl beta-glucan, a novel beta-glucan. *Food Chem Toxicol*, *152*, 112226. https://doi.org/10.1016/j.fct.2021.112226

Ramnath, R. D., Ng, S. W., Guglielmotti, A., & Bhatia, M. (2008). Role of MCP-1 in endotoxemia and sepsis. *Int Immunopharmacol*, *8*(6), 810-818. https://doi.org/10.1016/j.intimp.2008.01.033

Riedemann, N. C., Neff, T. A., Guo, R. F., Bernacki, K. D., Laudes, I. J., Sarma, J. V.,…Ward, P. A. (2003). Protective effects of IL-6 blockade in sepsis are linked to reduced C5a receptor expression. *J Immunol*, *170*(1), 503-507. https://doi.org/10.4049/jimmunol.170.1.503

Romero, C. R., Herzig, D. S., Etogo, A., Nunez, J., Mahmoudizad, R., Fang, G.,…Sherwood, E. R. (2010). The role of interferon-γ in the pathogenesis of acute intra-abdominal sepsis. *J Leukoc Biol*, *88*(4), 725-735. https://doi.org/10.1189/jlb.0509307

Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., Kievlan, D. R.,…Naghavi, M. (2020). Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet*, *395*(10219), 200-211. https://doi.org/10.1016/s0140-6736(19)32989-7

Saeed, S., Quintin, J., Kerstens, H. H., Rao, N. A., Aghajanirefah, A., Matarese, F.,…Stunnenberg, H. G. (2014). Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science*, *345*(6204), 1251086. https://doi.org/10.1126/science.1251086

Sheikh Motahar Vahedi, H., Bagheri, A., Jahanshir, A., Seyedhosseini, J., & Vahidi, E. (2019). Association of Lymphopenia with Short Term Outcomes of Sepsis Patients; a Brief Report. *Arch Acad Emerg Med*, *7*(1), e14.

Sheu, C. C., Gong, M. N., Zhai, R., Chen, F., Bajwa, E. K., Clardy, P. F.,…Christiani, D. C. (2010). Clinical characteristics and outcomes of sepsis-related vs non-sepsis-related ARDS. *Chest*, *138*(3), 559-567. https://doi.org/10.1378/chest.09-2933

Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M.,…Angus, D. C. (2016). The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama*, *315*(8), 801-810. https://doi.org/10.1001/jama.2016.0287

Soltys, J., & Quinn, M. T. (1999). Modulation of endotoxin- and enterotoxin-induced cytokine release by in vivo treatment with beta-(1,6)-branched beta-(1,3)-glucan. *Infect Immun*, *67*(1), 244-252. https://doi.org/10.1128/iai.67.1.244-252.1999

Sousa, N., Figueiredo, I., Araújo, M., Andrade, E., Alves, T., Silva, B.,…Fernandes, E. S. (2019). Punica granatum Leaf Lipophilic Fraction Favours Mortality by Attenuating Inflammation in Mice with Polymicrobial Sepsis. *Journal of Pharmacy and Pharmacology*, *7*. https://doi.org/10.17265/2328-2150/2019.07.002

Suffredini, A. F., Fromm, R. E., Parker, M. M., Brenner, M., Kovacs, J. A., Wesley, R. A., & Parrillo, J. E. (1989). The cardiovascular response of normal humans to the administration of endotoxin. *N Engl J Med*, *321*(5), 280-287. https://doi.org/10.1056/nejm198908033210503

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J.,…Mering, C. V. (2019). STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*, *47*(D1), D607-d613. https://doi.org/10.1093/nar/gky1131

Tatli Seven, P., Iflazoglu Mutlu, S., Seven, I., Arkali, G., Ozer Kaya, S., & Kanmaz, O. E. (2021). Protective role of yeast beta-glucan on lead acetate-induced hepatic and reproductive toxicity in rats. *Environ Sci Pollut Res Int*, *28*(38), 53668-53678. https://doi.org/10.1007/s11356-021-14398-0

Taveira da Silva, A. M., Kaulbach, H. C., Chuidian, F. S., Lambert, D. R., Suffredini, A. F., & Danner, R. L. (1993). Brief report: shock and multiple-organ dysfunction

after self-administration of Salmonella endotoxin. *N Engl J Med*, *328*(20), 1457-1460. https://doi.org/10.1056/nejm199305203282005

Tracey, K. J., Lowry, S. F., Fahey, T. J., 3rd, Albert, J. D., Fong, Y., Hesse, D.,…et al. (1987). Cachectin/tumor necrosis factor induces lethal shock and stress hormone responses in the dog. *Surg Gynecol Obstet*, *164*(5), 415-422.

van der Poll, T., Keogh, C. V., Guirao, X., Buurman, W. A., Kopf, M., & Lowry, S. F. (1997). Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia. *J Infect Dis*, *176*(2), 439-444. https://doi.org/10.1086/514062

van der Poll, T., van de Veerdonk, F. L., Scicluna, B. P., & Netea, M. G. (2017). The immunopathology of sepsis and potential therapeutic targets. *Nature Reviews Immunology*, *17*(7), 407-420. https://doi.org/10.1038/nri.2017.36

Vardon-Bounes, F., Gratacap, M. P., Groyer, S., Ruiz, S., Georges, B., Seguin, T.,…Minville, V. (2019). Kinetics of mean platelet volume predicts mortality in patients with septic shock. *PLoS One*, *14*(10), e0223553. https://doi.org/10.1371/journal.pone.0223553

Vardon-Bounes, F., Ruiz, S., Gratacap, M. P., Garcia, C., Payrastre, B., & Minville, V. (2019). Platelets Are Critical Key Players in Sepsis. *Int J Mol Sci*, *20*(14). https://doi.org/10.3390/ijms20143494

Venet, F., & Monneret, G. (2018). Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nat Rev Nephrol*, *14*(2), 121-137. https://doi.org/10.1038/nrneph.2017.165

Vetvicka, V., Vannucci, L., & Sima, P. (2020). β-glucan as a new tool in vaccine development. *Scand J Immunol*, *91*(2), e12833. https://doi.org/10.1111/sji.12833

Vincent, J. L., Sakr, Y., Singer, M., Martin-Loeches, I., Machado, F. R., Marshall, J. C.,…Angus, D. C. (2020). Prevalence and Outcomes of Infection Among Patients in Intensive Care Units in 2017. *Jama*, *323*(15), 1478-1487. https://doi.org/10.1001/jama.2020.2717

Vlassopoulou, M., Yannakoulia, M., Pletsa, V., Zervakis, G. I., & Kyriacou, A. (2021). Effects of fungal beta-glucans on health - a systematic review of randomized controlled trials. *Food Funct*, *12*(8), 3366-3380. https://doi.org/10.1039/d1fo00122a

Waage, A., Halstensen, A., & Espevik, T. (1987). Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. *Lancet*, *1*(8529), 355-357. https://doi.org/10.1016/s0140-6736(87)91728-4

Walachowski, S., Breyne, K., Secher, T., Cougoule, C., Guzylack-Piriou, L., Meyer, E.,…Tabouret, G. (2022). Oral supplementation with yeast β-glucans improves the resolution of Escherichia coli-associated inflammatory responses independently of monocyte/macrophage immune training. *Front Immunol*, *13*, 1086413. https://doi.org/10.3389/fimmu.2022.1086413

Wang, T., Dai, H., Wan, N., Moore, Y., & Dai, Z. (2008). The role for monocyte chemoattractant protein-1 in the generation and function of memory CD8+ T cells. *J Immunol*, *180*(5), 2886-2893. https://doi.org/10.4049/jimmunol.180.5.2886

Wzorek-Łyczko, K., Woźniak, W., Piwowarczyk, A., & Kuchar, E. (2024). The anti-infective effect of β-glucans in children. *Int J Vitam Nutr Res*, *94*(3-4), 296-307. https://doi.org/10.1024/0300-9831/a000793

Yin, K., Gribbin, E., & Wang, H. (2005). Interferon-gamma inhibition attenuates lethality after cecal ligation and puncture in rats: implication of high mobility group box-1. *Shock*, *24*(4), 396-401. https://doi.org/10.1097/01.shk.0000175556.03300.c6

Yin, K., Hock, C. E., Lai, P. S., Ross, J. T., & Yue, G. (1999). Role of interferon-gamma in lung inflammation following cecal ligation and puncture in rats. *Shock*, *12*(3), 215-221. https://doi.org/10.1097/00024382-199909000-00008

5. CONCLUSÕES

A administração com β-glucana demonstrou efeitos promissores em modelo de sepse letal, evidenciando seu potencial terapêutico. Em camundongos sépticos, a administração de β-glucana aumentou o número absoluto de linfócitos e granulócitos no sangue periférico, indicando uma estimulação da resposta imune. Além disso, reduziu o número de células na medula óssea, baço e peritônio, sugerindo um possível controle da infiltração celular excessiva nesses tecidos. A preservação da arquitetura pulmonar, sem alterações histológicas significativas, reforça seu papel protetor, embora tenha sido observada congestão leve a moderada nos vasos peribronquiolares. O tratamento também manteve o controle de citocinas desreguladas na sepse, como TNF-α, INF-γ, MCP-1 e IL-10, ao mesmo tempo em que estimulou a produção de IL-6, sem interferir na produção de IL-12. Adicionalmente, a β-glucana reduziu significativamente os níveis de unidades formadoras de colônias no peritônio e no sangue periférico, inibindo a translocação bacteriana e, consequentemente, aumentando a sobrevida dos animais sépticos. Diante desses achados, o tratamento com a β-glucana de *Auricularia auricula* pode ser considerado uma alternativa terapêutica para estimular e/ou modular a resposta imune em quadros de sepse, podendo também atuar como adjuvante ao tratamento com antimicrobianos padrões na clínica, contribuindo assim para a melhora do prognóstico e sobrevida na sepse.

6. REFERÊNCIAS

Akramiene, D., Kondrotas, A., Didziapetriene, J., & Kevelaitis, E. (2007). Effects of beta-glucans on the immune system. *Medicina (Kaunas, Lithuania)*, *43*, 597-606.

Al Tuwaijri, A. S., Mahmoud, A. A., Al Mofleh, I. A., & Al Khuwaitir, S. A. (1987). Effect of glucan on Leishmania major infection in BALB/c mice. *J Med Microbiol*, *23*(4), 363-365. https://doi.org/10.1099/00222615-23-4-363

Angus, D. C., Linde-Zwirble, W. T., Lidicker, J., Clermont, G., Carcillo, J., & Pinsky, M. R. (2001). Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med*, *29*(7), 1303-1310. https://doi.org/10.1097/00003246-200107000-00002

Babayigit, H., Kucuk, C., Sozuer, E., Yazici, C., Kose, K., & Akgun, H. (2005). Protective effect of β-glucan on lung injury after cecal ligation and puncture in rats. *Intensive Care Medicine*, *31*(6), 865-870. https://doi.org/10.1007/s00134-005-2629-x

Basso, A. M. M. (2017). *Modulação da resposta imune inata de polissacarídeos de basidiomicetos na infecção experimental de Cryptococcus neoformans* Universidade de Brasília (UnB)]. http://repositorio.unb.br/handle/10482/23541

Basso, A. M. M., De Castro, R. J. A., de Castro, T. B., Guimarães, H. I., Polez, V. L. P., Carbonero, E. R.,…Bocca, A. L. (2020). Immunomodulatory activity of β-glucan-containing exopolysaccharides from Auricularia auricular in phagocytes and mice infected with Cryptococcus neoformans. *Med Mycol*, *58*(2), 227-239. https://doi.org/10.1093/mmy/myz042

Bauer, B., & Steinle, A. (2017). HemITAM: A single tyrosine motif that packs a punch. *Sci Signal*, *10*(508). https://doi.org/10.1126/scisignal.aan3676

Behall, K. M., Scholfield, D. J., & Hallfrisch, J. (1997). Effect of beta-glucan level in oat fiber extracts on blood lipids in men and women. *Journal of the American College of Nutrition*, *16*(1), 46-51. https://doi.org/10.1080/07315724.1997.10718648

Bode, K., Bujupi, F., Link, C., Hein, T., Zimmermann, S., Peiris, D.,…Krammer, P. H. (2019). Dectin-1 Binding to Annexins on Apoptotic Cells Induces Peripheral Immune Tolerance via NADPH Oxidase-2. *Cell Rep*, *29*(13), 4435-4446.e4439. https://doi.org/10.1016/j.celrep.2019.11.086

Bone, R. C., Balk, R. A., Cerra, F. B., Dellinger, R. P., Fein, A. M., Knaus, W. A.,…Sibbald, W. J. (1992). Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest*, *101*(6), 1644-1655. https://doi.org/10.1378/chest.101.6.1644

Bonfim-Mendonça, P. D., Capoci, I. R., Tobaldini-Valerio, F. K., Negri, M., & Svidzinski, T. I. (2017). Overview of β-Glucans from Laminaria spp.: Immunomodulation Properties and Applications on Biologic Models. *International Journal of Molecular Sciences*, *18*(9).

Brown, G. D., Herre, J., Williams, D. L., Willment, J. A., Marshall, A. S. J., & Gordon, S. (2003). Dectin-1 Mediates the Biological Effects of β-Glucans. *Journal of Experimental Medicine*, *197*(9), 1119-1124. https://doi.org/10.1084/jem.20021890

Brown, G. D., Taylor, P. R., Reid, D. M., Willment, J. A., Williams, D. L., Martinez-Pomares, L.,…Gordon, S. (2002). Dectin-1 is a major beta-glucan receptor on macrophages. *J Exp Med*, *196*(3), 407-412. https://doi.org/10.1084/jem.20020470

Carbonero, E. R., Novikov, T. S. M., Gomes, Y. G. S., Brito, D. R., Coelho, L. C., Mendes, M. F.,…Lião, L. M. (2024). Chemical Characterization and In Vitro Evaluation of Glucans from Fermentation-Produced Nutraceutical Bionutri-AR1®: Antioxidant and Immunomodulatory Properties. *Pharmaceutics*, *16*(11), 1404.

Carcillo, J. A., & Shakoory, B. (2024). Cytokine Storm and Sepsis-Induced Multiple Organ Dysfunction Syndrome. *Adv Exp Med Biol*, *1448*, 441-457. https://doi.org/10.1007/978-3-031-59815-9_30

Cheng, Q. J., Farrell, K., Fenn, J., Ma, Z., Makanani, S. K., & Siemsen, J. (2024). Dectin-1 ligands produce distinct training phenotypes in human monocytes through differential activation of signaling networks. *Scientific Reports*, *14*(1), 1454. https://doi.org/10.1038/s41598-024-51620-8

Chihara, G., Maeda, Y., Hamuro, J., Sasaki, T., & Fukuoka, F. (1969). Inhibition of mouse sarcoma 180 by polysaccharides from Lentinus edodes (Berk.) sing. *Nature*, *222*(5194), 687-688. https://doi.org/10.1038/222687a0

Di Luzio, N., & Riggi, S. (1970). The effects of laminarin, sulfated glucan and oligosaccharides of glucan on reticuloendothelial activity. *Journal of the Reticuloendothelial Society*, *8*(5), 465-473.

dos Santos, J. V., Ribeiro Lima Lins de Araújo, M., Costa Marinho Toledo, M., Camerino Bomfim, L., Cavalcante Lessa, A. E., Araujo Ramos dos Santos, P. R.,…Alves da Silva, L. M. (2024). Análise Epidemiológica e tendências de mortalidade por sepse no Brasil de 2018 a 2022. *Brazilian Journal of Implantology and Health Sciences*, *6*(8), 5148-5161. https://doi.org/10.36557/2674-8169.2024v6n8p5148-5161

Evans, L., Rhodes, A., Alhazzani, W., Antonelli, M., Coopersmith, C. M., French, C.,…Levy, M. (2021). Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med*, *47*(11), 1181-1247. https://doi.org/10.1007/s00134-021-06506-y

Gour, N., Lajoie, S., Smole, U., White, M., Hu, D., Goddard, P.,…Wills-Karp, M. (2018). Dysregulated invertebrate tropomyosin-dectin-1 interaction confers susceptibility to allergic diseases. *Sci Immunol*, *3*(20). https://doi.org/10.1126/sciimmunol.aam9841

Guarino, M., Perna, B., Cesaro, A. E., Maritati, M., Spampinato, M. D., Contini, C., & De Giorgio, R. (2023). 2023 Update on Sepsis and Septic Shock in Adult Patients: Management in the Emergency Department. *J Clin Med*, *12*(9). https://doi.org/10.3390/jcm12093188

Gupta, M., Abu-Ghannam, N., & Gallaghar, E. (2010). Barley for Brewing: Characteristic Changes during Malting, Brewing and Applications of its By-Products. *Comprehensive Reviews in Food Science and Food Safety*, *9*(3), 318-328. https://doi.org/https://doi.org/10.1111/j.1541-4337.2010.00112.x

Han, M. D., Han, J., Hyun, S. H., & Shin, H. W. (2008). Solubilization of water-insoluble beta-glucan isolated from Ganoderma lucidum. *Journal of environmental biology*, *29*(2), 237.

Herre, J., Gordon, S., & Brown, G. D. (2004). Dectin-1 and its role in the recognition of beta-glucans by macrophages. *Mol Immunol*, *40*(12), 869-876. https://doi.org/10.1016/j.molimm.2003.10.007

ILAS, I. L. A. d. S.-. (2021). *GUIA PRÁTICO DE TERAPIA ANTIMICROBIANA NA SEPSE*. Retrieved 20 de dezembro de 2024 from https://ilas.org.br/wp-content/uploads/2022/02/Guia_ATM.pdf

Johansson, L., Virkki, L., Maunu, S., Lehto, M., Ekholm, P., & Varo, P. (2000). Structural characterization of water soluble β-glucan of oat bran. *Carbohydrate Polymers*, *42*(2), 143-148. https://doi.org/https://doi.org/10.1016/S0144-8617(99)00157-5

Kang, M. A., Jeon, Y. K., & Nam, M. J. (2020). Auricularia auricula increases an apoptosis in human hepatocellular carcinoma cells via a regulation of the peroxiredoxin1. *J Food Biochem*, *44*(10), e13373. https://doi.org/10.1111/jfbc.13373

Kaur, R., Sharma, M., Ji, D., Xu, M., & Agyei, D. (2020). Structural Features, Modification, and Functionalities of Beta-Glucan. *Fibers*, *8*(1), 1.

Keogh, G. F., Cooper, G. J. S., Mulvey, T. B., McArdle, B. H., Coles, G. D., Monro, J. A., & Poppitt, S. D. (2003). Randomized controlled crossover study of the effect of a highly β-glucan–enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men2. *The American Journal of Clinical Nutrition*, *78*(4), 711-718. https://doi.org/https://doi.org/10.1093/ajcn/78.4.711

Kong, X., Duan, W., Li, D., Tang, X., & Duan, Z. (2020). Effects of Polysaccharides From Auricularia auricula on the Immuno-Stimulatory Activity and Gut Microbiota in Immunosuppressed Mice Induced by Cyclophosphamide. *Front Immunol*, *11*, 595700. https://doi.org/10.3389/fimmu.2020.595700

Legese, M. H., Asrat, D., Swedberg, G., Hasan, B., Mekasha, A., Getahun, T.,…Mihret, A. (2022). Sepsis: emerging pathogens and antimicrobial resistance in Ethiopian referral hospitals. *Antimicrobial Resistance & Infection Control*, *11*(1), 83. https://doi.org/10.1186/s13756-022-01122-x

Lemieszek, M., & Rzeski, W. (2012). Anticancer properties of polysaccharides isolated from fungi of the Basidiomycetes class [journal article]. *Contemporary Oncology/Współczesna Onkologia*, *16*(4), 285-289. https://doi.org/10.5114/wo.2012.30055

Levy, M. M., Fink, M. P., Marshall, J. C., Abraham, E., Angus, D., Cook, D.,…Ramsay, G. (2003). 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med*, *31*(4), 1250-1256. https://doi.org/10.1097/01.ccm.0000050454.01978.3b

Liang, J., Melican, D., Cafro, L., Palace, G., Fisette, L., Armstrong, R., & Patchen, M. L. (1998). Enhanced clearance of a multiple antibiotic resistant Staphylococcus aureus in rats treated with PGG-glucan is associated with increased leukocyte counts and increased neutrophil oxidative burst activity. *Int J Immunopharmacol*, *20*(11), 595-614. https://doi.org/10.1016/s0192-0561(98)00007-1

Liu, D., You, M., Zhao, G. F., Li, X. J., Song, Y. X., Dou, H.,…Hou, Y. Y. (2017). The novel α-glucan YCP improves the survival rates and symptoms in septic mice by regulating myeloid-derived suppressor cells. *Acta Pharmacol Sin*, *38*(9), 1269-1281. https://doi.org/10.1038/aps.2017.27

Liu, Z., Li, L., Xue, B., Zhao, D., Zhang, Y., & Yan, X. (2021). A New Lectin from Auricularia auricula Inhibited the Proliferation of Lung Cancer Cells and Improved Pulmonary Flora. *Biomed Res Int*, *2021*, 5597135. https://doi.org/10.1155/2021/5597135

Lull, C., Wichers, H. J., & Savelkoul, H. F. (2005). Antiinflammatory and immunomodulating properties of fungal metabolites. *Mediators Inflamm*, *2005*(2), 63-80. https://doi.org/10.1155/mi.2005.63

Manners, D. J., Masson, A. J., & Patterson, J. C. (1973). The structure of a β-(1→3)-d-glucan from yeast cell walls. *Biochemical Journal*, *135*(1), 19-30. https://doi.org/10.1042/bj1350019

Mata-Martínez, P., Bergón-Gutiérrez, M., & Del Fresno, C. (2022). Dectin-1 Signaling Update: New Perspectives for Trained Immunity. *Front Immunol*, *13*, 812148. https://doi.org/10.3389/fimmu.2022.812148

McIntosh, M., Stone, B. A., & Stanisich, V. A. (2005). Curdlan and other bacterial (1→3)-β-d-glucans. *Applied Microbiology and Biotechnology*, *68*(2), 163-173. https://doi.org/10.1007/s00253-005-1959-5

Mithal, L. B., Arshad, M., Swigart, L. R., Khanolkar, A., Ahmed, A., & Coates, B. M. (2022). Mechanisms and modulation of sepsis-induced immune dysfunction in children. *Pediatr Res*, *91*(2), 447-453. https://doi.org/10.1038/s41390-021-01879-8

Moorlag, S., Khan, N., Novakovic, B., Kaufmann, E., Jansen, T., van Crevel, R.,…Netea, M. G. (2020). β-Glucan Induces Protective Trained Immunity against Mycobacterium tuberculosis Infection: A Key Role for IL-1. *Cell Rep*, *31*(7), 107634. https://doi.org/10.1016/j.celrep.2020.107634

Murphy, E. J., Rezoagli, E., Major, I., Rowan, N., & Laffey, J. G. (2021). β-Glucans. *Encyclopedia*, *1*(3), 831-847.

Pillemer, L., & Ecker, E. (1941). Anti-complementary factor in fresh yeast.

Rhodes, A., Evans, L. E., Alhazzani, W., Levy, M. M., Antonelli, M., Ferrer, R.,…Dellinger, R. P. (2017). Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Medicine*, *43*(3), 304-377. https://doi.org/10.1007/s00134-017-4683-6

Rowe, T. A., & McKoy, J. M. (2017). Sepsis in Older Adults. *Infect Dis Clin North Am*, *31*(4), 731-742. https://doi.org/10.1016/j.idc.2017.07.010

Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., Kievlan, D. R.,…Naghavi, M. (2020). Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet*, *395*(10219), 200-211. https://doi.org/10.1016/s0140-6736(19)32989-7

Sanitária, M. d. S.-A. N. d. V. (2018). *RESOLUÇÃO DA DIRETORIA COLEGIADA – RDC Nº 243, DE 26 DE JULHO DE 2018*. Retrieved from https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2018/rdc0243_26_07_2018.pdf

Schorey, J. S., & Lawrence, C. (2008). The pattern recognition receptor Dectin-1: from fungi to mycobacteria. *Curr Drug Targets*, *9*(2), 123-129. https://doi.org/10.2174/138945008783502430

Senoglu, N., Yuzbasioglu, M. F., Aral, M., Ezberci, M., Kurutas, E. B., Bulbuloglu, E.,…Ciragil, P. (2008). Protective Effects of N-Acetylcysteine and β -Glucan Pretreatment on Oxidative Stress in Cecal Ligation and Puncture Model of Sepsis. *Journal of Investigative Surgery*, *21*(5), 237-243. https://doi.org/10.1080/08941930802180136

Shear, M., Turner, F. C., Perrault, A., & Shovelton, T. (1943). Chemical treatment of tumors. V. Isolation of the hemorrhage-producing fraction from Serratia marcescens (Bacillus prodigiosus) culture filtrate. *J Natl Cancer Inst*, *4*(1), 81-97.

Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M.,…Angus, D. C. (2016). The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama*, *315*(8), 801-810. https://doi.org/10.1001/jama.2016.0287

Srivastava, H., Breuninger, E., Creech, H. J., & Adams, G. (1962). PREPARATION AND PROPERTIES OF POLYSACCHARIDE–LIPID COMPLEXES FROM SERRATIA MARCESCENS. *Canadian Journal of Biochemistry and Physiology*, *40*(7), 905-918.

Synytsya, A., & Novak, M. (2014). Structural analysis of glucans. *Ann Transl Med*, *2*(2), 17. https://doi.org/10.3978/j.issn.2305-5839.2014.02.07

Teas, J. (1982). The dietary intake of laminaria, a brown seaweed, and breast cancer prevention. *Nutrition and Cancer*, *4*(3), 217-222. https://doi.org/10.1080/01635588209513760

Torres, L. K., Pickkers, P., & van der Poll, T. (2022). Sepsis-Induced Immunosuppression. *Annual Review of Physiology*, *84*(Volume 84, 2022), 157-181. https://doi.org/https://doi.org/10.1146/annurev-physiol-061121-040214

Vincent, J. L. (2022). Current sepsis therapeutics. *EBioMedicine*, *86*, 104318. https://doi.org/10.1016/j.ebiom.2022.104318

Walachowski, S., Breyne, K., Secher, T., Cougoule, C., Guzylack-Piriou, L., Meyer, E.,…Tabouret, G. (2022). Oral supplementation with yeast β-glucans improves the resolution of Escherichia coli-associated inflammatory responses independently of monocyte/macrophage immune training. *Front Immunol*, *13*, 1086413. https://doi.org/10.3389/fimmu.2022.1086413

Wang, W., Ma, L., Liu, B., & Ouyang, L. (2024). The role of trained immunity in sepsis. *Front Immunol*, *15*, 1449986. https://doi.org/10.3389/fimmu.2024.1449986

Weyd, H., Abeler-Dörner, L., Linke, B., Mahr, A., Jahndel, V., Pfrang, S.,…Krammer, P. H. (2013). Annexin A1 on the surface of early apoptotic cells suppresses CD8+ T cell immunity. *PLoS One*, *8*(4), e62449. https://doi.org/10.1371/journal.pone.0062449

Whitehead, A., Beck, E. J., Tosh, S., & Wolever, T. M. (2014). Cholesterol-lowering effects of oat β-glucan: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*, *100*(6), 1413-1421. https://doi.org/10.3945/ajcn.114.086108

Williams, D., Al-Tuwaijri, A., & Di Luzio, N. (1980). Influence of glucan on experimental infections in mice. *International Journal of Immunopharmacology*, *2*(3), 189.

Williams, D. L., Yaeger, R. G., Pretus, H. A., Browder, I. W., McNamee, R. B., & Jones, E. L. (1989). Immunization against Trypanosoma cruzi: adjuvant effect of glucan. *Int J Immunopharmacol*, *11*(4), 403-410. https://doi.org/10.1016/0192-0561(89)90087-8

Xia, Y., Vetvicka, V., Yan, J., Hanikýrová, M., Mayadas, T., & Ross, G. D. (1999). The beta-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. *J Immunol*, *162*(4), 2281-2290.

Xu, N., Zhou, Y., Lu, X., & Chang, Y. (2021). Auricularia auricula-judae (Bull.) polysaccharides improve type 2 diabetes in HFD/STZ-induced mice by regulating the AKT/AMPK signaling pathways and the gut microbiota. *J Food Sci*, *86*(12), 5479-5494. https://doi.org/10.1111/1750-3841.15963

Yang, R., Sun, L., Li, C. F., Wang, Y. H., Yao, J., Li, H.,…Hung, M. C. (2021). Galectin-9 interacts with PD-1 and TIM-3 to regulate T cell death and is a target for cancer immunotherapy. *Nat Commun*, *12*(1), 832. https://doi.org/10.1038/s41467-021-21099-2

Yang, R. Y., Rabinovich, G. A., & Liu, F. T. (2008). Galectins: structure, function and therapeutic potential. *Expert Rev Mol Med*, *10*, e17. https://doi.org/10.1017/s1462399408000719

Zhu, Z., He, L., Bai, Y., Xia, L., Sun, X., & Qi, C. (2023). Yeast β-glucan modulates macrophages and improves antitumor NK-cell responses in cancer. *Clin Exp Immunol*, *214*(1), 50-60. https://doi.org/10.1093/cei/uxad080