Journal of Infection 90 (2025) 106391

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Review

Immune checkpoint blockade in experimental bacterial infections

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ARTICLE INFO

Article history:
Accepted 22 December 2024
Available online 3 January 2025

Keywords:
Immunotherapy
Immune checkpoint inhibitors
Immune checkpoint proteins
Bacterial infections
Animal model
Drug repurposing

SUMMARY

Immune checkpoint inhibitors designed to reinvigorate immune responses suppressed by cancer cells have revolutionized cancer therapy. Similarities in immune dysregulation between cancer and infectious diseases have prompted investigations into the role of immune checkpoints in infectious diseases, including the therapeutic potential of immune checkpoint blockade and drug repurposing. While most research has centered around viral infections, data for bacterial infections are emerging. This systematic review reports on the in vivo effect of immune checkpoint blockade on bacterial burden and selected immune responses in preclinical studies of bacterial infection, aiming to assess if there could be a rationale for using immunotherapy for bacterial infections. Of the 42 analyzed studies, immune checkpoint blockade reduced the bacterial burden in 60% of studies, had no effect in 28% and increased the bacterial burden in 12%. Findings suggest that the effect of immune checkpoint blockade on bacterial burden is context-dependent and in part relates to the pathogen. Further preclinical research is required to understand how the therapeutic effect of immune checkpoint blockade is mediated in different bacterial infections, and if immune checkpoint blockade can be used as an adjuvant to conventional infection management strategies.

Introduction

Inhibitory immune checkpoints are immune regulatory proteins expressed on the surface of immune cells and are responsible for maintaining self-tolerance and preventing excessive activation of the immune system.[1] Cancer cells can upregulate inhibitory immune checkpoints to suppress T-cell responses and escape immunosurveillance.[1] This occurs when immune checkpoint receptors (e.g. PD-1) expressed mainly on T-cells interact with their corresponding ligands (e.g. PD-L1 and PD-L2) expressed on cancer or antigen-presenting cells.[1] Immune checkpoint inhibitors designed to block this interaction and restore T-cell functionality to recognize and eliminate cancer cells has become a revolutionary approach in cancer immunotherapy, and today, antibody therapies targeting three immune checkpoint pathways (PD-1/PD-L1, CTLA-4, LAG-3) have been approved by the U.S. Food and Drug Administration for various types of cancer with hundreds of ongoing clinical trials.[1–3]

Given the similarities in immunosuppressive features of cancer and different infections, including upregulation of inhibitory immune checkpoints on immune cells,[4,5] research has subsequently expanded to the infectious disease area, especially in chronic viral infections where immune checkpoint blockade (ICB) has shown potential in preclinical studies.[6] For example, administering anti-PD-1 antibodies to simian immunodeficiency virus-infected rhesus macaques has been associated with enhanced T-cell functions, improved viral suppression and delay of viral rebound.[6,7] This opens the possibility for new applications of ICB and repurposing of immune checkpoint inhibitors for infectious diseases.[8] Identifying new therapeutic strategies for bacterial infections in particular is essential given the global burden of antimicrobial resistance.[9] It is estimated that the number of deaths attributable to antimicrobial resistance will increase by ~70% from 2022 to 2050 if further actions are not taken.[9] However, developing new antibiotics is challenging. Of the 57 candidates currently in the clinical pipeline, 32 target pathogens on the World Health Organization's priority list, and only 12 are considered innovative.[10] Therefore, there is an increased focus

Abbreviations: ARRIVE, animal research: reporting of in vivo experiments; CTLA-4, cytotoxic T lymphocyte associated protein 4; ICB, immune checkpoint blockade; LAG-3, lymphocyte activation gene 3; PD-1, programmed cell death 1; PD-L1, programmed death ligand 1; SYRCLE, systematic review center for laboratory animal experimentation; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIM-3, T cell immunoglobulin and mucin domain 3

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https://doi.org/10.1016/j.jinf.2024.106391

on developing alternatives to traditional antimicrobials, including immunomodulating agents.[10]

Thus far, bacterial sepsis is among the most studied bacterial infections in immune checkpoint research. Meta-analyses of preclinical sepsis models have reported increased survival rates after ICB,[11,12] however, the effect on bacterial clearance and immune-related outcomes were inconclusive.[12] To our best knowledge, this has yet to be systematically reviewed for other bacterial infections. Therefore, in this systematic review, we explored what is currently known about the effect of ICB on bacterial burden as the primary outcome and immune responses (selected immune cell populations, cytokines and apoptosis) as the secondary outcome in preclinical animal models of various bacterial infections. We also assessed the quality of studies to collectively determine if current evidence could provide a foundation for working towards ICB as a therapeutic approach for bacterial infections.

Methods

Systematic literature search

Web of Science, PubMed and Scopus databases were searched until July 2024 by one assessor and screened using Covidence (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia). The search strategy outlined by the PRISMA flow diagram in Fig. S1 and Table S1A-B aimed at identifying preclinical animal studies in which ICB was administered in connection to a bacterial challenge. Only studies that reported a bacterial burden outcome, focused on an inhibitory immune checkpoint target, and used a blocking antibody or checkpoint-derived fusion protein intervention were included. Data relating to the animal model, bacterial challenge, ICB intervention, and primary and secondary outcomes were extracted by two assessors.

Bacterial burden, assessed by culture, PCR and immunofluorescence methods, was the primary outcome and reported as "reduced", "no effect" or "increased" by ICB relative to a control. Isotype controls were selected if studies reported both untreated and isotype controls. Bacterial burden was assessed for the primary organ (Table 1A) of each study, which was selected based on the disease model and available data. If the bacterial burden in the primary organ was measured at multiple timepoints, the study was registered once per outcome category, irrespective of the number of measurements. This was done to ensure that variation in measurements within a study was represented while excluding repeat measures within the same category to avoid overrepresentation. The

Table 1A
Study characteristics relating to the animal model and bacterial challenge.

Reference		Animal model				Bacterial challenge			
Reference	Study	Strain/breed	Age	Sex	Disease model	Inoculum	Dose	Route	Primary organ[a]
Dadelahi 2023[41]	1	C57BL/6J	6–12 wk	M+F	Brucellosis	*Brucella melitensis*	1×10^5 CFU	IP	Spleen
Li 2023[42]	1	C57BL/6	6–8 wk	F	Pneumonia	*Chlamydia psittaci*	5×10^6 IFU	Intranasal	Lung
Frankhauser 2014[43]	1	C57BL/6	NR	NR	Genital tract infection	*Chlamydia trachomatis*	10^6 IFU	Transcervial	Uterus
Peng 2011[44]	1	BALB/c	6–8 wk	F	Genital tract infection	*Chlamydia muridarum*	2×10^4 IFU	Intravaginal	Vagina
Ka 2015[45]	1	C57BL/6	7 wk	F	Q fever	*Coxiella burnetii*	10^6 bacteria	IP	Lung
Triantafyllou 2021[46]	1	C57BL/6	8–12 wk	M	Sepsis	*Escherichia coli*	5×10^7 bacteria/20 g	IV	Blood
Go 2021[47]	1	C57BL/6J	8 wk	M	Gastritis	*Helicobacter felis*	2×10^8 CFU/ml	Oral	Stomach
Anderson 2006[48]	1	C57BL/6	6–10 wk	F	Gastritis	*Helicobacter pylori*	NR	Gavage	Stomach
Watanabe 2004[49]	1	C57BL/6	6 wk	F	Gastritis	*Helicobacter pylori*	5×10^7 CFU/ml	GI	Stomach
Xu 2013[50]	1	C57BL/6	NR	NR	Listeriosis	*Listeria monocytogenes*	1×10^5 CFU	IV	Spleen
Xu 2013[50]	2	C57BL/6	NR	NR	Listeriosis	*Listeria monocytogenes*	1×10^5 CFU	IV	Spleen
Pedicord 2011[51]	1	C57BL/6J	NR	NR	Listeriosis	*Listeria monocytogenes*	10^5 CFU	IV	Spleen
Rowe 2009[52]	1	C57BL/6	6–8 wk	F	Listeriosis	*Listeria monocytogenes*	10^6 bacteria	IV	Spleen
Rowe 2008[53]	1	C57B6	6–8 wk	F	Listeriosis	*Listeria monocytogenes*	10^6 bacteria	IV	Spleen
Seo 2007[54]	1	C57BL/6	8–10 wk	NR	Listeriosis	*Listeria monocytogenes*	3000 CFU	IV	Spleen
McCulloch 2024[55]	2	C57BL/6J	8–12 wk	F	Tuberculosis	*Mycobacterium tuberculosis*	20–50 CFU	Aerosol	Lung
Qu 2024[56]	1	C57BL/6	NR	NR	Tuberculosis	*Mycobacterium tuberculosis*	2×10^6 CFU	IV	Lung
Qu 2024[56]	2	C57BL/6	NR	NR	Tuberculosis	*Mycobacterium tuberculosis*	2×10^6 CFU	IV	Lung
Kauffman 2021[25]	1	Rhesus macaque	2 y	M	Tuberculosis	*Mycobacterium tuberculosis*	30–50 CFU	BI	Lung
Kamboj 2020[57]	1	BALB/c	6–8 wk	NR	Tuberculosis	*Mycobacterium tuberculosis*	50–100 CFU	Aerosol	Lung
Jayaraman 2016[58]	1	C57BL/6J	6–8 wk	NR	Tuberculosis	*Mycobacterium tuberculosis*	200 CFU	Aerosol	Lung
Jayaraman 2010[59]	1	C57BL/6J	6–10 wk	NR	Tuberculosis	*Mycobacterium tuberculosis*	100–200 CFU	Aerosol	Lung
Kirman 1999[21]	1	C57BL/6	6–9 wk	M	Pneumonia	*Mycobacterium bovis* BCG	5×10^4–10^5 bacteria	Intranasal	Lung
Zhong 2024[60]	1	C57BL6/J	8–10 wk	M	Sepsis	Polymicrobial	-	CLP	PLF
Liu 2022[61]	1	C57BL/6	8–10 wk	M	Sepsis	Polymicrobial	-	CLP	Blood
Sun 2021[62]	1	C57BL/6J	6 wk	M+F	Sepsis	Polymicrobial	-	CLP	PLF
Zhao 2021[63]	1	NR	8–10 wk	M	Sepsis	Polymicrobial	-	CLP	PLF
Lou 2020[64]	1	C57BL/6	8–10 wk	M	Sepsis	Polymicrobial	-	CLP	Blood
Deng 2018[65]	1	C57BL/6J	8–10 wk	M+F	Sepsis	Polymicrobial	-	CLP	Blood
Zhang 2010[66]	1	C57BL/6	8–10 wk	M	Sepsis	Polymicrobial	-	CLP	Blood
Luo 2024[67]	1	C57BL/6	6–8 wk	F	Pneumonia	*Pseudomonas aeruginosa*	5×10^6 CFU	IT	Lung
Zhang 2019[68]	1	C57BL/6J	22–28 mo	M	Pneumonia	*Pseudomonas aeruginosa*	4×10^8 CFU/ml	IT	BAL
Patil 2018[69]	2	BALB/c	10–12 wk	M	Burn infection	*Pseudomonas aeruginosa*	1×10^6 CFU	Topical	Blood
Yang 2016[70]	1	C3H/HeN	6–7 wk	M	Far eastern spotted fever	*Rickettsia heilongjiangensis*	1×10^7 CFU	IP	Spleen
McCulloch 2024[55]	1	C57BL/6J	8–12 wk	F	Salmonella	*Salmonella enterica*	1×10^6 CFU	IP	Spleen
Johanns 2010[28]	1	129SvJxC57BL/6	6–8 wk	NR	Salmonella	*Salmonella enterica*	1×10^4 CFU	IV	Spleen
Terasaki 2024[71]	1	C57BL/6	8–20 wk	M+F	MRSA infection	*Staphylococcus aureus*	1×10^9 CFU/ml	IV	Blood
Yang 2024[72]	1	C57BL/6	8–12 wk	M+F	Osteomyelitis	*Staphylococcus aureus*	2×10^5 CFU/ml	Trauma	Bone
Li 2023[26]	1	C57BL/6	10–12 wk	NR	Osteomyelitis	*Staphylococcus aureus*	1×10^5 CFU/ml	Trauma	Bone
Li 2023[26]	2	C57BL/6	10–12 wk	NR	Osteomyelitis	*Staphylococcus aureus*	1×10^5 CFU/ml	Trauma	Bone
Curran 2021[73]	1	C57BL/6J	12 wk	F	Pneumonia	*Staphylococcus aureus*	8.5×10^9 CFU/kg	IT	Lung
Patil 2018[69]	1	BALB/c	10–12 wk	M	Burn infection	*Staphylococcus aureus*	1×10^8 CFU	IV	Lung

Abbreviations: BI: bronchoscopically instilled, CFU: colony forming units, CLP: cecal ligation and puncture, IFU: inclusion forming units, IP: intraperitoneal, IT: intratracheal, IV: intravenous, GI: gastric intubation, MRSA: methicillin-resistant *Staphylococcus aureus*, NR: not reported, PLF: peritoneal lavage fluid.
[a] Used when reporting the bacterial burden outcome.

log change in bacterial burden between intervention and control groups was estimated by extracting central tendency values from graphs using PlotDigitalizer v3 (https://plotdigitizer.com/).[13]

Secondary outcomes were immune checkpoint expression after bacterial challenge, immune cell populations ($CD4^+$T-cells and $CD8^+$T-cells), cytokines (pro- and anti-inflammatory) and apoptosis. These were the most reported outcomes across studies. Immune checkpoint expression after bacterial challenge was limited to the immune checkpoint targeted by the intervention. Immune outcomes were also reported as "reduced", "no effect" or "increased" by ICB relative to a control. Immune outcomes were assessed using multiplex immunoassays, flow cytometry, ELISA, PCR, histological, colorimetric and automated blood counting methods. Only immune outcomes assessed directly on cells/tissue from the animals were included, and measurements from ex vivo/in vitro stimulated cells were therefore excluded. Measurements on all tissues/cells were included for immune outcomes. If the immune outcome was measured at multiple timepoints or in multiple tissues/cells, the study was registered once per outcome category, irrespective of the number of measurements. Cytokines were grouped as pro- or anti-inflammatory according to Turner et al.[14] and assessed collectively in each study. Data for secondary outcomes were only extracted if there was a statistical comparison between the intervention and control group. The present review is descriptive, and data are presented as number or percentage of studies or as log differences, and graphs were created using GraphPad Prism version 10.3.1 (GraphPad Software, Massachusetts, USA).

Bias assessment

Bias was evaluated independently by two assessors using a modified version of SYRCLE's risk of bias tool for animal studies,[15,16] which was adapted to fit this research field. Modifications included appropriate baseline characteristics (strain/breed, age/weight, sex), assessment of bias according to the primary outcome, addition of antibiotic co-administration as a domain, and changes to some answer categories (Table S2). Consensus between assessors was reached by discussion.[15]

Results

Study characteristics

The systematic search identified 2155 articles, of which 37 articles met the inclusion criteria and comprised 42 individual studies. Most of these articles have been published within the last decade (Fig. S2). Tables 1A and 1B summarize data relating to the animal model, bacterial challenge and ICB intervention. Of the 42 studies, 41 were performed in mice and 1 in monkeys. Animals were inoculated with 16 different bacterial strains belonging to 11 genera, i.e. *Brucella* (2% of studies), *Chlamydia* (7%), *Coxiella* (2%), *Escherichia* (2%), *Helicobacter* (7%), *Listeria* (14%), *Mycobacterium* (19%), *Pseudomonas* (7%), *Rickettsia* (2%), *Salmonella* (5%), *Staphylococcus* (14%), or were subjected to cecal ligation and puncture resulting in a polymicrobial infection (17%). Immune checkpoint targets included CTLA-4 (14%), LAG-3 (2%), PD-1 (19%), PD-L1 (42%), TIGIT (9%), and TIM-3 (14%). In 39 studies, antibodies were used to block the immune checkpoint pathway, while three studies used a checkpoint-derived fusion protein. The intervention was administered prophylactically in 54% of studies and as a treatment in 46% of studies. Isotype antibody, IgG or Fab controls were used in all but three studies, that used untreated or PBS controls.

Immune checkpoint expression after bacterial challenge

Fifteen studies reported the expression of immune checkpoints after bacterial challenge. Compared to uninfected controls, immune checkpoint expression was upregulated in at least one tissue/cell population after bacterial challenge in all studies. In seven studies (17%), immune checkpoints were upregulated in all reported cells/tissues, whereas the expression was tissue/cell or time-dependent in eight studies (19%). Immune checkpoint expression was either insufficiently reported (no comparison to an uninfected control group or no statistics) or not reported at all in 27 studies (64%) (Fig. 1A).

Bacterial burden

No studies reported complete bacterial eradication, although some showed significant reductions in bacterial burden following ICB. The log reduction in bacterial burden ranged from 0.1 to 3.2 (Fig. 1B). Overall, ICB reduced the bacterial burden in 60% of studies, whereas it had no effect in 28% and increased the bacterial burden in 12% of studies (Fig. 1C). At a genus level, polymicrobial infection, *Pseudomonas* and *Escherichia* had the best outcomes, whereas *Brucella*, *Coxiella*, *Listeria* and *Rickettsia* had the worst (Fig. 1D). Results were similar between immune checkpoint targets apart from CTLA-4, which had the least effect on bacterial burden (Fig. 1E). A higher percentage of studies reported reduced bacterial burden when administering the intervention as a treatment (85%) than prophylactically (41%) (Fig. 1F).

Immune parameters

The effect of ICB on selected immune outcomes ($CD4^+$T-cell and $CD8^+$T-cell populations, pro- and anti-inflammatory cytokines or apoptosis) was reported in 24 studies. $CD4^+$T-cells and $CD8^+$T-cells were increased by ICB in 44% and 31% of studies, respectively, while 31% of studies reported a reduction in $CD8^+$T-cells by ICB. Studies assessed seven pro-inflammatory (IL-1β, IL-1α, IL-6, IL-17/A, IL-18, IFN-γ, TNF/-α) and three anti-inflammatory (IL-10, IL-12/p40/p70, IL-22) cytokines.[14] The changes in pro- and anti-inflammatory cytokines after ICB were similar, with the proportion of studies reporting an increase, no effect and reduction of pro-inflammatory cytokines being 38%, 44%, and 19%, respectively, while this was 44%, 33% and 22% for anti-inflammatory cytokines. ICB reduced apoptosis in 63% of studies (Fig. 2). A detailed description of these immune outcomes for each study is listed in Table S3-5.

Study quality

Relevant baseline characteristics were reported in 70% of studies. In 22% of studies, randomization of the intervention was reported, but only three studies described the timing, and none described methodological details of randomization. Randomized housing was not reported in any studies. Blinding was only performed in two studies. While 81% of studies reported n-values for the primary outcome, it was unclear if the dataset was complete in 46% of cases, and no studies reported a sample size calculation. In eight studies, the intervention was administered alongside antibiotics, which could have influenced the outcomes (Fig. 3). Studies not administering antibiotics were therefore reported as having sufficient methodology in Fig. 3.

Discussion

In this review, analyzing 42 preclinical animal studies of bacterial infections, ICB reduced the bacterial burden in 60% of studies, while 28% showed no effect and 12% increased the bacterial burden. The most promising results were seen for polymicrobial infections, *Pseudomonas* and *Escherichia.* On the contrary, for some pathogens that can cause intracellular infections like *Brucella, Coxiella*, *Listeria* and *Rickettsia*, ICB had no effect or even increased the bacterial burden. Intracellular pathogens may have developed pathogen-

Table 1B
Study characteristics relating to the immune checkpoint intervention.

Reference		Immune checkpoint blockade						Co-administration
Reference	Study	Target	Dose[a]	Route	Indication	Time relative to inoculation[b]	Control	
Pedicord 2011[51]	1	CTLA-4	200 µg	IP	Prophylactic	2 h pre-ino	Isotype AB	-
Johanns 2010[28]	1	CTLA-4	500 µg (+250 µg)	IP	Treatment	5, 8 d or 37, 40 d post-ino	Isotype AB	-
Rowe 2009[52]	1	CTLA-4	500 µg (+250 µg)	IP	Prophylactic	1 d pre-ino + 4, 8 d post-ino	Isotype AB	-
Anderson 2006[48]	1	CTLA-4[c]	200 µg	IP	Prophylactic	1 d pre-ino + q2d	Fab control	-
Watanabe 2004[49]	1	CTLA-4	100 µg	IP	Prophylactic	1 d pre-ino + q1d for 7 d	IgG control	-
Kirman 1999[21]	1	CTLA-4	1 mg	IP	Prophylactic	With ino + q1w	Untreated	-
Lou 2020[64]	1	LAG-3	50 µg	IP	Treatment	3 h post-ino	Isotype AB	Imipenem
Qu 2024[56]	1	PD-1	250 µg	IP	Treatment	3, 10, 17 d post-ino	Isotype AB	-
Dadelahi 2023[41]	1	PD-1	250 µg	IP	Prophylactic	1 d pre-ino + q3d	Isotype AB	-
Li 2023[26]	1	PD-1	200 µg	IP	Treatment	5 d post-ino + q3d	Isotype AB	Gentamicin
Kauffman 2021[25]	1	PD-1	10 mg/kg	IV	Treatment	2,4,6,8,10,13 wk post-ino	Isotype AB	-
Triantafyllou 2021[46]	1	PD-1	200 µg	IP	Prophylactic	1 d pre-ino	Isotype AB	-
Kamboj 2020[57]	1	PD-1	200 µg/kg	IP	Treatment	q4–5d post-ino (3 times)	Isotype AB	Rifampicin
Zhang 2019[68]	1	PD-1	200 µg	IV	Prophylactic	With ino	Isotype AB	Gentamicin
Xu 2013[50]	1	PD-1	200 µg	IP	Prophylactic	1 d pre-ino + q2d	IgG control	-
Luo 2024[67]	1	PD-L1	200 µg	IP	Prophylactic	1 d pre-ino	Untreated	-
Terasaki 2024[71]	1	PD-L1	200 µg	IV	Prophylactic	With ino	Isotype AB	-
Qu 2024[56]	2	PD-L1	250 µg	IP	Treatment	3, 10, 17 d post-ino	Isotype AB	-
Yang 2024[72]	1	PD-L1	200 µg	IP	Treatment	1 d post-ino + q2d for 2 wk	Isotype AB	Gentamicin
Li 2023[26]	2	PD-L1	200 µg	IP	Treatment	5 d post-ino + q3d	Isotype AB	Gentamicin
Curran 2021[73]	1	PD-L1	300 µg	IP	Prophylactic	With ino + 1 d post-ino	Isotype AB	-
Go 2021[47]	1	PD-L1	300 µg	IP	NR	q3d[d]	Isotype AB	-
Zhao 2021[63]	1	PD-L1	2.5 mg/kg	IV	Treatment	3 h post-ino	Isotype AB	-
Deng 2018[65]	1	PD-L1	20 mg/kg	NR	Treatment	3, 24, 48 h post-ino	IgG control	-
Patil 2018[69]	1	PD-L1	200 µg	IP	Prophylactic	1 d pre-ino	Isotype AB	-
Patil 2018[69]	2	PD-L1	50 µg	IP	Prophylactic	1 d pre-ino	Isotype AB	-
Ka 2015[45]	1	PD-L1	200 µg	NR	Prophylactic	1 d pre-ino	Isotype AB	-
Frankhauser 2014[43]	1	PD-L1	200 µg	NR	Prophylactic	1,2,3 d pre-ino + q2d post-ino	Isotype AB	-
Xu 2013[50]	2	PD-L1	200 µg	IP	Prophylactic	1 d pre-ino + q2d	IgG control	-
Peng 2011[44]	1	PD-L1+TIM-3	200 µg +100 µg	IP	Prophylactic	With ino + 2, 4 d post-ino (TIM-3) With ino + 3,6,9,12 d post-ino (PD-L1)	Isotype AB	-
Zhang 2010[66]	1	PD-L1	50 µg	IP	Treatment	3 h post-ino	Isotype AB	-
Rowe 2008[53]	1	PD-L1	500 µg (+250 µg)	IP	Prophylactic	1 d pre-ino + 4, 8 d post-ino	Isotype AB	-
Seo 2007[54]	1	PD-L1	200 µg	IP	Prophylactic	1 d pre-ino[e]	Isotype AB	-
Li 2023[42]	1	TIM-3	100 µg	IP	Treatment	2, 4, 6, 8, 10 d post-ino	Isotype AB	-
Liu 2022[61]	1	TIM-3	50 µg	IV	Treatment	30 min post-ino	Isotype AB	Imipenem
Jayaraman 2016[58]	1	TIM-3	500 µg (+100 µg)	IP	Treatment	10 wk post-ino + q3d for 2 wk	Isotype AB	-
Yang 2016[70]	1	TIM-3[c]	200 µg	IP	Prophylactic	12 h pre-ino	Ig control	-
Jayaraman 2010[59]	1	TIM-3[c]	0.5 mg (+0.1 mg)	IP	Treatment	1,5,8,12 d post-ino	IgG control	-
McCulloch 2024[55]	1	TIGIT[f]	200 µg	IP	Treatment	1 d post-ino + q3d	Isotype AB	-
McCulloch 2024[55]	2	TIGIT[f]	200 µg	IP	Treatment	Twice weekly from 2 wk post-ino	Isotype AB	-
Zhong 2024[60]	1	TIGIT	400 µg	IP	Prophylactic	1 d pre-ino	PBS	-
Sun 2021[62]	1	TIGIT	400 µg	IP	Treatment	12 h and 24 h post-ino	Isotype AB	Ceftriaxone and Metronidazole

Abbreviations: AB: antibody, CTLA-4: cytotoxic T lymphocyte-associated protein 4, Fab: fragment antigen-binding region, IP: intraperitoneal, IV: intravenous, LAG-3: lymphocyte activation gene 3, NR: not reported, PBS: phosphate-buffered saline, PD-1: programmed cell death 1, PD-L1: programmed death ligand 1, TIGIT: T cell immunoreceptor with Ig and ITIM domains, TIM-3: T cell immunoglobulin and mucin domain 3.

[a] Numbers in brackets indicate follow-up doses.
[b] Pre-, with or post-inoculation, q3d = every 3 days.
[c] Uses a checkpoint-derived fusion protein instead of an antibody intervention.
[d] Unclear when the intervention was first administered.
[e] For immune-related outcomes, the intervention was given 1 d pre ino + 2 d post ino.
[f] WT antibody was assessed.

specific virulence factors or mechanisms to evade the immune system and survive within host cells that may not be targeted by ICB, which could render enhanced T-cell responses insufficient and in part explain the lack of effect.[17,18] Notably, some of these pathogens were only reported by few studies and findings thus need to be confirmed. This data suggest that the efficacy of ICB in part depends on the pathogen, which is similar to what has been reported for cancer ICB therapy, where the response rate varies significantly between different cancers.[19]

Blocking inhibitory immune checkpoint pathways can reinvigorate proliferation and effector functions of T-cells.[20] Here, expansion of $CD4^+$ and $CD8^+$T-cell populations was reported in less than half of the studies. Surprisingly, 25–56% of studies reported no effect across the selected immune outcomes. This could relate to antibody affinity, expression level of the targeted immune checkpoint or measurements in off-target tissues/cells.[21] Immune checkpoints are also found on innate immune cells like macrophages, dendritic cells and NK-cells.[20] Recent literature suggests that blocking some classic immune checkpoint pathways as well as phagocytosis checkpoint pathways, like SIRPα/CD47, can directly or indirectly modulate the innate immune response, which is also important for combatting bacterial infections, and should be explored further.[20,22,23] The heterogenicity of studies prevented further comparisons of immune cells in this review.

While preclinical data for certain pathogens are encouraging, the progression of ICB therapy for bacterial infections is challenged by fundamental outstanding questions. First, the identification of key immune checkpoints in different pathologies and why ICB treatment responses vary remains to be determined.[5,24] For example, among *Listeria monocytogenes* studies, PD-1, but not PD-L1 or CTLA4 blockade, was able to reduce the bacterial burden despite similarities in study design. Such

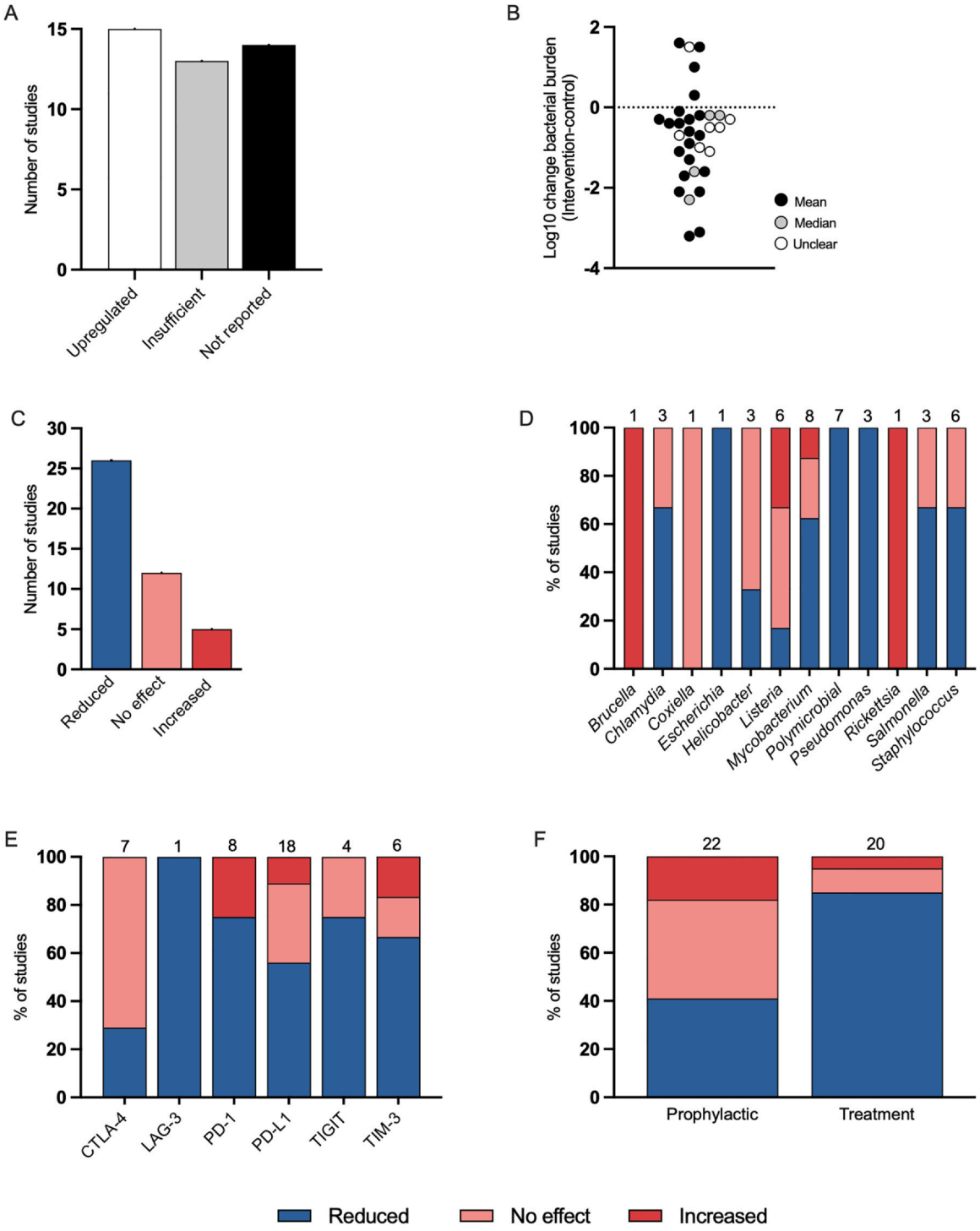

Fig. 1. Immune checkpoint expression and bacterial burden outcomes. Immune checkpoint expression after bacterial challenge compared to uninfected controls **(A)**. Log change in bacterial burden between intervention and control groups **(B)**. Effect of immune checkpoint blockade on bacterial burden in the primary organ across all studies **(C)**, according to bacterial genus **(D)**, according to immune checkpoint target **(E)** and according to indication **(F)** compared to controls. In (A), upregulated refers to upregulation of the immune checkpoint in at least one reported tissue or cell population, and insufficient refers to lack of statistics or an uninfected control group. In (B) colored dots refer to the central tendency values used. Seven studies did not specify the central tendency value. Numbers above the graphs indicate the number of studies that the data originates from.

subgroup explorations for other bacterial infections are currently limited by heterogenicity in study design or a low number of studies but are important for understanding context-dependent effects of ICB on bacterial burden. Additionally, some studies report large inter-animal variability in the response to ICB, with non-responders that are comparable to controls,[25,26] despite the ability to control for many variables in animal experiments, including the use of inbred mouse strains. Second, understanding which stage of infection to intervene with ICB is important.[27] Here, we report that a higher proportion of studies were associated with reduced bacterial burden when ICB was administered as a treatment than prophylactically. The effect may also differ over the course of infection, as demonstrated for *Salmonella enterica,* where CTLA-4 blockade was effective in reducing the bacterial burden in the acute, but not chronic stage of infection.[28] Third, the translatability of

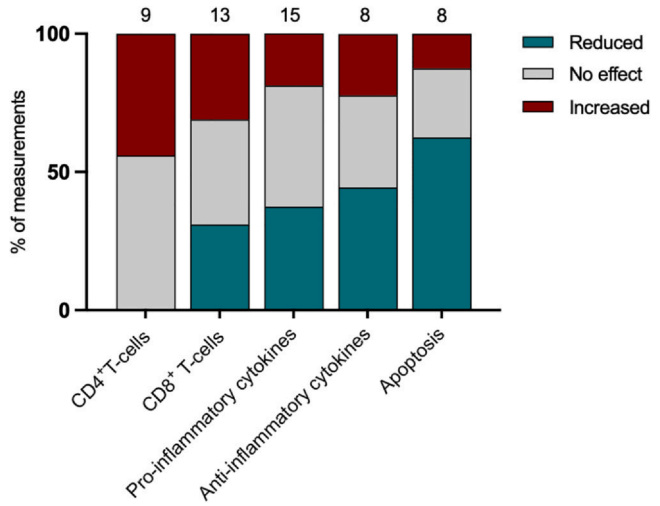

Fig. 2. Immune-related outcomes. Effect of immune checkpoint blockade on $CD4^+$T-cell and $CD8^+$T-cell populations, pro- and anti-inflammatory cytokines and apoptosis compared to controls. Both general and antigen-specific $CD4^+$ and $CD8^+$ T-cell populations are reported. Numbers above the graph indicate the number of studies that the data originates from.

preclinical animal studies in this field remains unclear. Most studies were performed in mice. Dawson, et al. reported a similarity in immune-related proteins between mice and humans of 73%, which is significantly lower than other experimental animals like pigs,[29] and elements of the immune response to bacterial infections in mice are different to humans. For example, circulating immune cells are dominated by lymphocytes in mice and neutrophils in humans, and some chemokines involved in host defenses in humans, such as IL-8, have not been identified in mice.[14,29,30] Additionally, variation in susceptibility and immune responses to bacterial infections have been demonstrated between mouse strains.[31,32] Although the molecular structure of mouse PD-L1 is similar to that of humans, and can form a functional immune checkpoint with human PD-1, it is unclear how well ICB of PD-L1 in mice is predictive of outcomes in humans.[33] This relates to observed differences in druggability between human and mouse PD-L1 for anti-human antibodies, peptides and small molecules.[33] Here, only one of five PD-L1 targeted molecules was able to block the mouse PD-L1/human PD-1 interaction but all five blocked the human PD-L1/human PD-1 interaction in an in vitro cell assay.[33] Such inter-species comparisons are lacking for several other immune checkpoint molecules. These factors may affect the translation of immunotherapies from mouse models to humans. Fourth, few studies reported co-administration of ICB and antibiotics. In cancer patients, antibiotic administration has been negatively correlated to the efficacy of ICB therapy.[34] Therefore, studies addressing the efficacy and compatibility of ICB and antibiotics in bacterial infections are needed.[4,12] Fifth, safety of ICB is a concern. As most infections are associated with inflammation, ICB may exacerbate this inflammatory response, and lead to immune-related adverse events.[27] Furthermore, it is noteworthy that some studies report an increase in bacterial burden following ICB. This is a relevant perspective not only for the therapeutic potential and safety of ICB in bacterial infections but also for cancer patients who are undergoing ICB therapy. It has been suggested that infections following ICB therapy in cancer patients may be a result of hyperinflammation induced by ICB that disrupts the balance between pathogen control and avoidance of immunopathology, favoring bacterial growth.[35] Additionally, it has been suggested that immune checkpoint pathways may be necessary to establish latent infections like *Mycobacterium tuberculosis*, and that ICB thus can cause re-activation of the infection.[35] ICB may also enhance T-cell mediated lysis of infected cells, which could promote bacterial dissemination.[36] Sixth, included studies display heterogenicity in terms of study design, animal models and bacterial strains which can influence the comparability of results and highlights the need for generating more standardized protocols in future research. Finally, most studies did not report methods used to reduce potential bias and may be underpowered. This is important as results from preclinical studies may be used as a basis for clinical studies.[12] The ARRIVE guidelines for reporting animal research address bias, and compliance with these guidelines is now a requirement for many scientific journals and will hopefully improve the quality and reproducibility of future studies in this field.[37]

Conclusion and future perspectives

In conclusion, 42 preclinical animal studies have investigated the effect of ICB on bacterial burden across different bacterial infections. In 60% of the studies, ICB successfully reduced the bacterial burden in a context-dependent manner that in part relates to the pathogen. Ongoing preclinical research is essential to understand how the therapeutic effect of ICB in bacterial infections may relate to different pathogens, immune checkpoint targets and timing of treatment initiation. Furthermore, to determine if ICB could be used as an adjuvant to conventional infection management strategies. To improve the translatability of such research, animal models with higher immune system homology to humans and in which the structure, function and druggability of immune checkpoints resemble that of humans should ideally be used. Additionally, analyses of tissue biopsies from patients with both acute and chronic infections would be beneficial for identifying relevant immune checkpoint targets across different bacterial infections. This might also clarify if a more personalized approach, as seen in oncology, should be considered. Finally, preclinical studies should include strategies to mitigate bias.

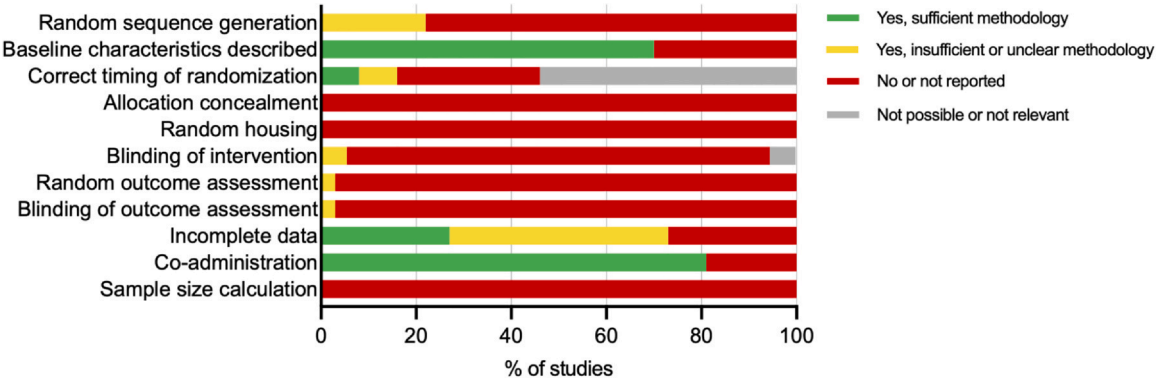

Fig. 3. Bias assessment. Bias assessment of 37 articles, comprising 42 studies, on immune checkpoint blockade in preclinical animal models of bacterial infection using a modified version of SYRCLE's risk of bias tool for animal studies.

Clinical translation

The findings of this review have seen only limited clinical translation which underscores the field's novelty. However, to succeed, future clinical studies must rely on a stronger preclinical foundation than identified by this review. Just three clinical trials of ICB in bacterial infections have been conducted so far focusing on the safety, pharmacokinetics and pharmacodynamics of anti-PD-1 and anti-PD-L1 therapy in patients with sepsis-induced immunosuppression. One study was a phase 1b randomized, double-blind, placebo-controlled, ascending-dose study of anti-PD-L1 therapy (BMS-936559) involving 24 participants. This study found that ICB was well tolerated at drug doses of 10–900 mg, did not induce hypercytokinemia, and was associated with increased monocytic human leukocyte antigen-DR expression at higher doses indicating improved immune function.[38] The mortality rate in this study was 25% and 4 participants experienced serious adverse events, none of which were considered drug related.[38] Another phase 1b randomized, double-blind study of anti-PD-1 therapy (Nivolumab) in 31 participants reported a mortality rate of 39% across both tested doses (480 mg and 960 mg), and 5 participants experienced adverse events that were possibly drug related. The authors concluded that the safety findings were consistent with the current drug label, and there was no indication of hypercytokinemia.[39] Finally, an open-label phase 1/2 study of anti-PD-1 therapy (Nivolumab) in 13 participants reported good tolerability and safety at doses of 480 mg and 960 mg. Here, the mortality rate was 31%, and one participant developed drug-related adverse events.[40] Both studies involving Nivolumab demonstrated an increase in monocytic human leukocyte antigen-DR expression over time, however, the findings were limited by the absence of a placebo group. Studies aimed at investigating the clinical efficacy of ICB for bacterial infections are lacking. However, the overall findings of this review indicate that ICB has therapeutic potential for certain types of bacterial infections, which warrants further investigation.

Funding

The work was supported by the Novo Nordisk Foundation NNF20SA0064340 (NLH).

CRediT authorship contribution statement

LKJ: Conceptualization, Methodology, Formal analysis, Writing – review & editing. **NLH:** Conceptualization, Methodology, Formal analysis, Visualization, Funding acquisition, Writing – original draft, Writing – review & editing. **PØJ:** Methodology, Formal analysis, Writing – review & editing.

Data availability

Data that support the findings of this study are available in the Supplementary Material and at https://figshare.com/s/83d64671bd47d8cc8f41.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106391.

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