Title

Therapeutic evaluation of immunomodulators in reducing surgical wound infection

Journal

The FASEB Journal, 36(1)

ISSN

0892-6638

Authors

Mahmud, Foyez

Roy, Ruchi

Mohamed, Mohamed F

et al.

Publication Date

2022

DOI

10.1096/fj.202101019r

Peer reviewed

HHS Public Access

Author manuscript

FASEB J. Author manuscript; available in PMC 2023 January 01.

Published in final edited form as:

FASEB J. 2022 January ; 36(1): e22090. doi:10.1096/fj.202101019R.

Therapeutic Evaluation of Immunomodulators in Reducing Surgical Wound Infection

Foyez Mahmud[1,2,#], **Ruchi Roy**[1,2,#], **Mohamed F. Mohamed**[1,2], **Anahita Aboonabi**[1,2], **Mario Moric**[3], **Kamran Ghoreishi**[5], **Mohammad Bayat**[6,7], **Timothy M. Kuzel**[1,2], **Jochen Reiser**[1], **Sasha H. Shafikhani**[1,2,4,*]

[1]Department of Medicine, Rush University Medical Center, Chicago, IL, USA

[2]Division of Hematology/Oncology/Cell Therapy, Rush University Medical Center, Chicago, IL, USA

[3]Department of Anesthesiology, Rush University Medical Center, Chicago, IL, USA

[4]Cancer Center, Rush University Medical Center, Chicago, IL, USA

[5]Department of Statistics, Qom University, Qom, Iran

[6]Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran

[7]Price Institute of Surgical Research, University of Louisville and Noveratech LLC. of Louisville, Louisville, KY, USA

Abstract

Despite many advances in infection control practices, including prophylactic antibiotics, surgical site infections (SSIs) remain a significant cause of morbidity, prolonged hospitalization, and death worldwide. Our innate immune system possesses a multitude of powerful antimicrobial strategies which make it highly effective in combating bacterial, fungal, and viral infections. However, pathogens use various stealth mechanisms to avoid innate immune system, which in turn buy them time to colonize wounds and damage tissues at surgical sites. We hypothesized that immunomodulators that can jumpstart and activate innate immune responses at surgical sites, would likely reduce infection at surgical sites. We used three immunomodulators; fMLP (formyl-Methionine-Lysine-Proline), CCL3 (MIP-1α), and LPS (Lipopolysaccharide), based on their documented ability to elicit strong inflammatory responses; in a surgical wound infection model with *Pseudomonas aeruginosa* to evaluate our hypothesis. Our data indicate that one-time topical treatment with these immunomodulators at low doses significantly increased proinflammatory responses in infected and uninfected surgical wounds and were as effective, (or even better),

*To whom correspondence should be addressed: Sasha_Shafikhani@rush.edu.
#These authors contributed to the manuscript equally

AUTHOR CONTRIBUTIONS: S. H. S. conceived and coordinated all aspects of the studies and wrote the paper. F.M conducted and contributed to Figures 1-7. R.R conducted and contributed to Figures 1-5 and 7. M.F.M. contributed Figures 1, 4, 6, and 7. A.A., contributed to Figures 4 & 6. M.M., conducted statistical analyses for all figures. S.K.G., contributed to statistical analysis in Figure 4. M.B., contributed to data analyses and research design, and T.M.K. and J.R. contributed to data analyses and research design, and reagents.

CONFLICT OF INTEREST: Dr. Sasha Shafikhani is the inventor on an approved patent (PCT/US2019041112), filed by Rush University Medical Center.

than a potent prophylactic antibiotic (Tobramycin) in reducing *P. aeruginosa* infection in wounds. Our data further show that immunomodulators did not have adverse effects on tissue repair and wound healing processes. Rather, they enhanced healing in both infected and uninfected wounds. Collectively, our data demonstrate that harnessing the power of innate immune system by immunomodulators can significantly boost infection control and potentially stimulate healing. We propose that topical treatment with these immunomodulators at the time of surgery may have therapeutic potential in combating SSI, alone or in combination with prophylactic antibiotics.

Keywords

Wound infection; Wound healing; Surgical Site Infection (SSI); Pseudomonas aeruginosa; Immunomodulators; fMLP (fMLF); CCL3 (MIP-1α); Lipopolysaccharides (LPS); Innate Immune System; Leukocytes; Neutrophils

INTRODUCTION

Various infection prevention measures have been used to reduce surgical site infection (SSI), including surgical hand asepsis, reduction of foot traffic in and out of the operating room, use of intraoperative skin antiseptic agents, perioperative high inspired oxygen, perioperative glycemic control, appropriate selection of surgical dressings, and perioperative antibiotic prophylaxis (1-9). Despite these preventive measures, SSI remains one of the most common and important healthcare-associated infections, accounting for 17-20% of all hospital-acquired infections (10-14). The Centers for Disease Control and Prevention (CDC) estimates that approximately 500,000 SSIs occur annually in the United States (US). Each SSI case is associated with approximately 7-11 additional postoperative hospital days (15-17), and 2-11-times higher risk of death compared with the operative patients without SSI (15, 18-22). The annual costs for Healthcare Associated Infections (HAIs) are astronomical and increasing every year, with SSIs contributing the most (~33.7%) to the overall costs, approximately $3.4 billion in US and between $3.5 to $10 billion in Europe (15, 16). Without question, SSI remains an important public health threat. It is not surprising that the US Department of Health and Human Services has identified combatting SSI as a top national priority.

Administration of antibiotic prophylaxis in the perioperative period, (~1 hour before surgery for most antibiotics), is the standard of care for most surgical procedures - although in some cases, (e.g., cardiac surgeries), post-surgical antibiotic for up to 3 days is recommended (18, 21-23). While antibiotics have saved millions of lives over the past 9 decades, their use is not without its problems. Excessive antibiotic use can lead to emergence of antibiotic resistance, increased risk for *Clostridium difficile* infection, cytotoxicity, allergic reactions, and immunological and neurological diseases - many of which have been attributed to dysbiosis in the gut flora (24-29). These limitations highlight the need for new approaches (preferably antibiotic-free) to enhance infection control at surgical sites.

We have evolved a remarkable and powerful innate immune system that recognizes invading pathogens as “none-self” and mobilizes its plethora of antimicrobial defenses to protect us against infection (30-36). Germline-encoded Pattern Recognition Receptors (PRRs) are at

the heart of innate immune system sensory and processing centers. Recognition of microbial pathogens by PRRs, such as toll-like receptors (TLRs), sets in motion a signaling cascade that culminates in the production of proinflammatory cytokines, such as TNF-α and IL-1β (30-35). These inflammatory cytokines function as powerful chemoattractants for effector innate immune leukocytes which destroy invading pathogens by various direct or indirect mechanisms (30-36). Neutrophils are the first effector inflammatory leukocytes infiltrating at the site of injury. In addition to their antimicrobial functions through phagocytosis, reactive oxygen species (ROS), neutrophil extracellular trap (NET) production, and antimicrobial peptides (AMPs) (37, 38), they also express various proinflammatory cytokines, such as IL-1β, which set the stage for the subsequent inflammatory responses, including monocytes recruitment and their differentiation into M1 classical macrophage phenotype which further contributes to microbial killing and infection control (39-43). Although, there is a high degree of redundancy in innate immune system's ability to sense and respond to invading pathogens, critical components of the innate immune system must function in an orderly fashion to eventually clear infection. For instance, mutations in PRRs, inflammasomes, or even individual inflammatory cytokines can render humans or animals vulnerable to infection (44-55). Further highlighting the importance of neutrophils and innate immune system in combating infection, Granulocyte Colony Stimulating Factor (G-CSF) and Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF) cytokines - which belong to a group of growth factors termed "Colony Stimulating Factors" which support survival, clonal expansion, and differentiation of hematopoietic myeloid progenitor cells (56-59) - have been approved for systemic use and shown to be effective in boosting infection control in patients suffering from neutropenia (reviewed in (60)).

Although neutrophil response begins immediately after injury and/or in response to infection and healthy immune system is effective in controlling infection for the most part, neutrophil and inflammatory responses reach their peak at 1-3 days after injury, depending on presence or absence of infection (61-63). This lag period in reaching the peak innate immune responses could potentially render wound tissues at surgical sites vulnerable to bacterial colonization at least early after surgery. In addition, pathogens have evolved many stealth virulence strategies that allow them to establish infection by dampening host's immune responses even in immunocompetent healthy individuals (64-68). These virulence stealth strategies utilized by pathogens, could further delay immune responses from reaching their peaks, thus extending the vulnerability period to infection in wounded tissues after surgery.

We hypothesized that immunomodulators that can jumpstart and activate innate immune responses at surgical sites, would likely enhance tissue's ability to fight off infection at surgical sites. SSIs include superficial incisional wound infections, infections of the deep incision space, and infections of organ space (22, 69, 70). We assessed the efficacy of immunomodulator-based approaches in reducing SSI in a surgical full-thickness incisional wound infection model against *Pseudomonas aeruginosa,* which is one of the most common and serious causes of wound and surgical site infections, both in healthy and immunocompromised individuals (71-75). We selected 3 immunomodulators with broad proinflammatory activities to evaluate their effectiveness in reducing *P. aeruginosa* infection in this model. We chose fMLP (*N*-formyl-Methionine-Leucyl-Phenylalanine; a.k.a., fMLF) because it primarily functions as a potent activator of phagocytic leukocytes (particularly

neutrophils) through its interaction with formyl peptide chemokine receptors (FPRs), although it has also been implicated in triggering the production of proinflammatory cytokines through activation of TLR2 and TLR4 (76-80). Of note, fMLP is a natural immunomodulator which is released from injured tissues, although it can also be released from invading bacterial pathogens (63, 81). CCL3 (a.k.a., MIP-1α) was chosen because it is an important proinflammatory cytokine which has been shown to recruit and activate phagocytic leukocytes (e.g., neutrophils), maintain the effector immune responses, and stimulate wound healing by engaging multiple chemokine receptors, such as CCR1, CCR4, and CCR5 (82-86). We also chose LPS (Lipopolysaccharide) because it is a bacterial ligand and a potent immunomodulator that triggers inflammatory responses primarily through TLR4, but it has also been implicated in triggering inflammatory responses through TLR2 and non-canonical caspase-11 inflammasome (80, 87). Our data indicate that one-time topical treatment with these immunomodulators at low doses significantly increased proinflammatory responses in infected and uninfected surgical wounds and were as effective, (or even better), than a potent prophylactic antibiotic (Tobramycin) in reducing *P. aeruginosa* infection in wounds. Moreover, treatment with these immunomodulators did not adversely impact wound healing processes in infected or uninfected wounds.

MATERIALS AND METHODS

CONTACT FOR REAGENT AND RESOURCE SHARING:

Further information and requests for reagents may be directed to, and will be fulfilled by, the Lead Contact, Sasha Shafikhani (Sasha_Shafikhani@rush.edu).

PROCEDURES RELATED TO ANIMAL STUDIES:

We have approval from the Rush University Medical Center Institutional Animal Care and Use Committee (IACUC No: 18-037 & 20-042) to conduct the research as indicated in these studies. All procedures complied strictly with the standards for care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, MD, USA). We obtained 6-7-week-old C57BL/6 and TLR4$^{-/-}$ knockout mice (Stock #029015) from the Jackson Laboratories (Bar Harbor, ME). These Mice were allowed to acclimate to the environment for 1 week prior to experimentation. Wounding and wound infection were carried out as we described previously (88) (71, 89, 90). We used *Pseudomonas aeruginosa* PA103 for this study, which has been described previously (91, 92) and we have shown that it causes massive infection and exacerbates wound damage in diabetic wounds (71). Immunomodulators were added at indicated levels right after wounding and prior to infection. Bacteria were prepared overnight as we described (92, 93). Infection levels in wounds were evaluated by determining the number of bacteria, colony forming unit (CFU) per gram of wound tissues, as described (6, 7, 71, 94, 95).

WOUNDING AND SURGICAL SITE INFECTION/TREATMENTS:

Full-thickness excisional wounding was performed, using sterile biopsy punches (5-mm diameter, AcudermH Inc. Lauterdale, FL), as we had described previously (71, 89, 90). Each animal received 4 equidistanced wounds on its back below the shoulder blades.

fMLP (a.k.a., fMLF), CCL3 (a.k.a., MIP-1α), and LPS immunomodulators (at indicated concentrations) were added once topically to the wound, right after wounding surgical procedure and prior to infection. Wound tissues from wound edges (~1 mm) were collected and analyzed in these studies as we described previously (89, 90, 95). For antibiotic prophylaxis, Tobramycin solution (0.35 mg/mL) or saline control were administered intraperitoneally (i.p.) in 0.2 mL saline, 1 hour before starting surgery, as described (96, 97).

HISTOPATHOLOGICAL EVALUATION:

Wound healing was assessed by digital photography, as described (71, 89, 90). Briefly, wound areas were determined by ImageJ at indicated timepoints. Wound closure rates were assessed by dividing the wound area at indicated timepoints to wound area at Day 0 (day of wound surgery). Leukocytes' infiltration in the wound bed were performed using hematoxylin and eosin (H&E) staining as described previously (6, 71). The number of leukocytes in wounds were assessed by determining the number of polymorphonuclear and mononuclear round cells which stained positive with eosin as described (71, 89). Neutrophil contents in wounds were assessed by anti-Ly6G histological analysis (6) and activated neutrophil contents in wounds were assessed by Myeloperoxidase (MPO) measurements, using ELISA (98). Macrophage levels in wounds were assessed by anti-CD68 histological analysis (89, 90).

REAGENTS:

Hematoxylin & Eosin Staining (Richard Allan Scientific Hematoxylin, Eosin Y, and Bluing Reagent Cat. Numbers: 7111L, 7211L, and 7301L from Thermo Fisher; anti-CD68 (Cat. No. NBP2-33337) and anti-Ly-6G (Cat. No. NBP2-00441) antibodies were purchased from Novus biologicals, CO. Myeloperoxidase (MPO) Mouse ELISA Kit (Cat. No., EMMPO) was obtained from Invitrogen; Lipopolysaccharides (LPS) was obtained from Sigma (Cat. No. L3012); CCL3 (rhCCL3/MIP-1α isoform LD78a; Cat. No., 450-MA/CF) was obtained from R&D; *N*-formyl-Met-Leu-Phe (fMLP, a.k.a., fMLF), Cat. No. 59880-97-6 from Sigma; Mouse IL-1β uncoated ELISA kit (Cat. No., 88-7013-88) and Mouse TNF-α uncoated ELISA kit (Cat. No., 88-7324-88) (ThermoFisher). Collagenase D, CAS No. 9001-12-1 (Sigma Aldrich).

STATISTICAL ANALYSES:

All variables were evaluated for violations of model assumptions. Distributions were evaluated descriptively using box plots, histograms, and quantile-quantile plots. If substantial skewness was discovered appropriate transformations were applied (i.e., square root). If outliers were identified, then sensitivity analyses were performed (with and without the outlier) and the more conservative result selected. Non-parametric methods were used if violations could not be appropriately compensated. T-tests were used for simple two group tests with homoscedasticity evaluated and (Satterthwaite approximation) adjustments made if necessary. Multi-group comparisons were performed using General Linear Models with Dunnett adjustment to compensate for error inflation for the contrasts between variables. Time varying data were analyzed using repeated measures (Proc Mixed) models with "Day" as the time varying factor with an autoregressive lag one covariance structure. Models were

evaluated for fit and multiple contrasts were adjusted for error inflation using the step-down Bonferroni (Holm) method. Data are represented using Mean ± SEM, figures plots were produced using GraphPad Prism version 5.0 and all statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). P-values less than or equal to 0.05 were considered as significant.

RESULTS

Treatment with immunomodulators boost innate immune responses in wound.

Full-thickness incisional wounds were generated on the back of C57B mice, as described (71, 89), and immediately treated with PBS (control) or PBS containing fMLP (5ng or 50ng per wound), CCL3 (1μg per wound), or LPS (10ng or 100ng per wound), prior to infection with *P. aeruginosa strain* PA103, which we have shown to establish robust infection and cause damage in diabetic wounds (71). Wound tissues were harvested at 24h after treatment and infection and assessed for their IL-1β and TNF-α proinflammatory cytokines by Enzyme-Linked Immunosorbent Assay (ELISA). Consistent with our hypothesis, immunomodulators were able to significantly boost the production of these proinflammatory cytokines in PA103-infected wounds, particularly when applied at higher concentrations (Fig. 1A-B).

We next evaluated the impact of immunomodulators (fMLP at 50ng, CCL3 at 1μg, and LPS at 100ng per wound) on inflammatory leukocytes' migration into wounds, 24h after treatment and infection by histological analysis, using hematoxylin and eosin (H&E) staining, as described (6, 89). In line with increased proinflammatory cytokines (Fig. 1), CCL3 and LPS immunomodulators also significantly enhanced leukocytes migration into wounds (Fig. 2A-B). fMLP treatment also increased leukocytes numbers in wound but the differences did not reach statistical significance. Phagocytic leukocytes (namely macrophages and neutrophils) play critical roles in combating invading pathogens in wound and at surgical sites (42, 99-101). We evaluated the impact of immunomodulators on macrophage and neutrophil responses in these wound by histological analyses using their specific markers CD68 and Ly6G respectively (89, 102). Data indicated that treatments with CCL3 and LPS immunomodulators significantly increased both macrophage and neutrophil contents of these wounds (Fig. 2C-F). Consistent with the leukocyte response, fMLP treatment also increased neutrophil and macrophage contents in infected wounds but the differences did not reach statistical significance. To further corroborate these data, we assessed neutrophil activation in wound using myeloperoxidase (MPO) - a marker primarily used for activated neutrophils (6, 103) - by ELISA. Data indicated that treatment with all 3 immunomodulators significantly increased activated neutrophils in infected wounds (Fig. 2G). Collectively, these data indicated that topical treatment with these immunomodulators enhanced inflammatory responses in infected wounds, albeit to different degree.

Immunomodulators enhance infection control in wound.

We next assessed the efficacy of Tobramycin antibiotic prophylaxis in controlling *P. aeruginosa* infection in this wound infection model, as a way to evaluate how immunomodulator-based therapies may compare in their ability to control infection with

a conventional antibiotic prophylaxis therapy. Tobramycin is a powerful antibiotic against *P. aeruginosa* infections, and it has been shown to be effective even against Gentamycin resistant *P. aeruginosa* clinical strains (104-106). We administered Tobramycin at 3.5 mg/kg by intraperitoneal injection (i.p), prophylactically at 1 hour prior to wounding and infection with PA103 strain at 10^3 or 10^6 bacteria/wound and assessed infection burden in wounds by colony forming unit (CFU) and bacteria counts determination as described (6, 7, 71). Dosing of Tobramycin was determined from previous publications, based on its efficacy to control infection without causing adverse side effects, such as nephrotoxicity or ototoxicity (97, 107-109). Tobramycin (Tob) prophylaxis therapy was very effective and reduced *P. aeruginosa* infection significantly by ~1 log-order, when 10^3 PA103 was used to infect (Fig. 3A), and by ~1.3 log order, when 10^6 PA103 was used to infect (Fig. 3E). Of note, no bacteria was detected in PBS-treated wounds, indicating that wound environment is effective in preventing low level infection with environmental bacteria (Fig. 3A, E).

We next assessed the effectiveness of immunomodulators in controlling infection by treating wounds with the aforementioned immunomodulators (fMLP at 50ng, CCL3 at 1μg, and LPS at 100ng per wound) prior to infection with 10^3 or 10^6 PA103. Data indicated that immunomodulators reduced infection by 1-1.3 log order, when 10^3 PA103 was used to infect (Fig. 3B-C), and by ~2-2.8 log order, when 10^6 PA103 was used to infect (Fig. 3F-H). These data demonstrated that these immunomodulators are at least as effective as systemic Tobramycin prophylactic antibiotic in reducing infection in wound.

Immunotherapy-induced enhancement in infection control is dependent on immune responses.

We reasoned that if our hypothesis is correct that immunomodulators enhance infection control by boosting innate immune responses in wound tissue, in situations where innate immune responses are not available and cannot be enhanced, immunomodulators should lose their effectiveness. Unlike fMLP and CCL3 which can stimulate inflammatory responses by engaging multiple receptors (76-80, 82-86), LPS primarily activates immune responses by engaging toll-like receptor 4 (TLR4) (80). To assess the dependence of LPS therapy on TLR4-mediated inflammatory responses, we evaluated the impact of LPS treatment on proinflammatory cytokines production, neutrophil activation, and infection control in $TLR4^{-/-}$ knockout mice. Consistent with our hypothesis, LPS treatment did not boost TNF-α and IL-1β production, or neutrophil activation in $TLR4^{-/-}$ infected wounds (Fig. 4A-C). Importantly, LPS-treated $TLR4^{-/-}$ wounds contained ~2.4 log-order more bacteria than LPS-treated C57B normal wounds, indicating that LPS-induced enhanced infection control in wound is primarily dependent on its ability to trigger inflammatory responses by engaging TLR4 receptor (Fig. 4D). Of note, $TLR4^{-/-}$ wounds contained significantly more bacteria (~0.8 log-order) than C57B normal wounds, indicating that TLR4 plays an important role in the recognition and inflammatory responses to *P. aeruginosa* infection (Fig. 4E).

Immunomodulators do not adversely affect healing in infected wounds.

Exuberant and persistent inflammation has been shown to be a major impediment to tissue repair and wound healing in chronic wounds (110-112). Although these immunomodulators

were effective in reducing infection, there remained a possibility that their use could lead to exuberant inflammation, which could harm tissue repair and adversely impact healing processes. To assess the potential long-term harmful side-effects of immunomodulator-based therapies on tissue repair, we assessed healing in wounds treated with immunomodulators or PBS and infected with PA103 (10^6) by digital photography, as described (71, 89). Data indicated that not only treatment with immunomodulators did not harm healing processes, they modestly but significantly improved healing in infected wounds (Fig. 5A-F).

Immunomodulators enhance proinflammatory responses in uninfected wound without harming healing processes.

Majority of surgical sites do not become infected (113, 114). Therefore, for immunomodulators to have therapeutic value, they must not adversely affect healing processes in uninfected wounds. To assess the impact of immunomodulators on healing processes in uninfected wounds, we first assessed inflammatory responses in uninfected wounds treated with fMLP (50ng/wound), CCL3 (1μg/wound), or LPS (100ng/ wound). Data indicated that treatment with CCL3 and LPS significantly increased IL-1β, TNF-α, and MPO proinflammatory markers in uninfected wounds (Fig. 6A-C). fMLP-treated uninfected wounds showed trends toward higher IL-1β and TNF-α contents but the differences did not reach statistical significance, as compared to PBS treated wounds (Fig. 6A-B). Of note, fMLP treatment significantly increased activated neutrophil contents (MPO) in uninfected wounds (Fig. 6C). Corroborating these data, CCL3 and LPS immunomodulators also increased leukocytes contents in uninfected wounds and similar to infected wounds, fMLP treatment increased leukocyte contents in uninfected wounds, although the differences did not reach statistical significance (Fig. 6D-E). These data indicated that these immunomodulators can boost inflammatory responses in uninfected wounds, albeit to different degree. We next assessed the impact of immunomodulators on wound healing in uninfected wounds. Similar to infected wounds, treatment with these immunomodulators did not harm healing processes, rather, they also modestly improved healing in uninfected wounds (Fig. 7).

DISCUSSION

We set out to examine whether treatment with immunomodulators - that can boost and direct innate immune responses at wound surgical sites - would be able to reduce infection at surgical wound site. For this purpose, we chose three proinflammatory immunomodulators; namely, fMLP, CCL3, and LPS, based on their documented ability to elicit strong inflammatory responses (63, 76-81, 87). Our data show that one-time topical treatment with these immunomodulators at very low doses were as effective, (if not better), as prophylactic Tobramycin in reducing *P. aeruginosa* infection in wound. Encouragingly, our data indicate that these immunomodulators not only did not adversely affect tissue repair and healing processes in infected or uninfected wounds, but they also improved wound healing, albeit modestly. Given that inflammation, aside from its role in combating invading pathogens, plays a critical role in wound healing processes (39, 115), our data suggest that immunomodulators may be able to accelerate healing processes even in the absence of infection by jumpstarting inflammatory responses.

Harnessing host innate immune powers by immunomodulators to control SSI has several advantageous over prophylactic antibiotics, although they could also potentially be administered in combination with antibiotics to further enhance their effectiveness. First, it is highly unlikely for a pathogen to develop resistance to all antimicrobial weapons that our immune system has at its disposal, including; phagocytosis, bursts of reactive oxygen species (ROS), hypochlorous acid (HOCl), neutrophil extracellular traps (NET), and antimicrobial peptides (AMPs) which are our own natural antibiotics with diverse structures and activities against viral, fungal, and bacterial pathogens (37-43, 116-119). Second, the choice of antibiotic prophylaxis is empirical and is determined based on the most probable cause of infection at the particular surgical site (21, 120). Prophylactic antibiotics could fail if the patient encounters a different pathogen or a pathogen that is resistant to the administered antibiotic (121). In contrast, immunomodulator-based therapies would not be empirically based because they mobilize innate immune system at surgical sites which in theory should be effective against majority of infections regardless of their origin (bacterial, fungal, or viral), due to the plethora of antimicrobial defenses at the disposal of innate immune system as discussed above. Third, immunomodulators likely have fewer undesirable side effects and may be safer than prophylactic antibiotics. For example, it is unlikely that topical use of immunomodulators would result in development of resistance, akin to antibiotic resistance, or lead to gut dysbiosis, as many antibiotics do (24-29).

Healthy people are expected to have normal immune responses, which for the most part are effective in protecting us against infection. Therefore, one might question the therapeutic value of using immunomodulators in healthy people with intact immune system. We posit that normal immune function does not necessarily mean optimal immune function which is needed to repel infection after surgery for the following reasons. First, inflammatory leukocytes' migration (such as neutrophil influx) reach their peak between 1-3 days after injury, depending on whether or not infection is present, although they begin migrating into the wound site immediately after injury (61-63). This lag period before inflammatory leukocytes reach their peak can potentially leave tissues at surgical sites unprotected and vulnerable to infection. In addition, many pathogens have evolved stealth virulence strategies that dampen host's immune responses, further delaying the immune function from reaching its optimal peak and allowing these pathogens to establish infection even in immunocompetent healthy individuals (64-68). Immunomodulators could potentially overcome at least some of these stealth strategies and shorten the period needed for immune function to reach its peak, thus fortifying tissue's defenses against invading pathogens.

In conclusion, we provide evidence that immunomodulators with the ability to mobilize and direct inflammatory responses at surgical site in wound, can enhance surgical site tissue defenses against infection without adversely impacting healing processes and tissue repair. Future studies should look to optimize immunomodulator-based therapies by increasing their levels without harmful side-effects, and by combining them with each other or with prophylactic antibiotics.

ACKNOWLEDGEMENTS:

We would like to thank the rest of Shafikhani lab members for their valued opinions on these studies. This work was supported by the National Institutes of Health (NIH) grants R01AI150668 and RO1DK107713, both to (S.H.S.).

REFERENCES

1. Edmiston CE Jr., Bruden B, Rucinski MC, Henen C, Graham MB, and Lewis BL (2013) Reducing the risk of surgical site infections: does chlorhexidine gluconate provide a risk reduction benefit? Am J Infect Control 41, S49–55 [PubMed: 23622749]
2. Panahi P, Stroh M, Casper DS, Parvizi J, and Austin MS (2012) Operating room traffic is a major concern during total joint arthroplasty. Clin Orthop Relat Res 470, 2690–2694 [PubMed: 22302655]
3. Sidhwa F, and Itani KM (2015) Skin preparation before surgery: options and evidence. Surg Infect (Larchmt) 16, 14–23 [PubMed: 25761076]
4. Campbell J, Filardo G, Bruce B, Bajaj S, Friel N, Hakimiyan A, Wood S, Grumet R, Shafikhani S, Chubinskaya S, and Cole BJ (2014) Salvage of contaminated osteochondral allografts: the effects of chlorhexidine on human articular chondrocyte viability. Am J Sports Med 42, 973–978 [PubMed: 24518877]
5. Greif R, Akca O, Horn EP, Kurz A, Sessler DI, and Outcomes Research G (2000) Supplemental perioperative oxygen to reduce the incidence of surgical-wound infection. N Engl J Med 342, 161–167 [PubMed: 10639541]
6. Kroin JS, Li J, Goldufsky JW, Gupta KH, Moghtaderi M, Buvanendran A, and Shafikhani SH (2016) Perioperative high inspired oxygen fraction therapy reduces surgical site infection with *Pseudomonas aeruginosa* in rats. Journal of medical microbiology 65, 738–744 [PubMed: 27302326]
7. Kroin JS, Buvanendran A, Li J, Moric M, Im H-J, Tuman KJ, and Shafikhani SH (2015) Short-term glycemic control is effective in reducing surgical site infection in diabetic rats. Anesthesia & Analgesia 120, 1289–1296 [PubMed: 25695673]
8. Stannard JP, Volgas DA, McGwin G 3rd, Stewart RL, Obremskey W, Moore T, and Anglen JO (2012) Incisional negative pressure wound therapy after high-risk lower extremity fractures. J Orthop Trauma 26, 37–42 [PubMed: 21804414]
9. Anesi JA, Blumberg EA, and Abbo LM (2018) Perioperative antibiotic prophylaxis to prevent surgical site infections in solid organ transplantation. Transplantation 102, 21–34 [PubMed: 28614192]
10. Magill SS, Hellinger W, Cohen J, Kay R, Bailey C, Boland B, Carey D, de Guzman J, Dominguez K, Edwards J, Goraczewski L, Horan T, Miller M, Phelps M, Saltford R, Seibert J, Smith B, Starling P, Viergutz B, Walsh K, Rathore M, Guzman N, and Fridkin S (2012) Prevalence of healthcare-associated infections in acute care hospitals in Jacksonville, Florida. Infect Control Hosp Epidemiol 33, 283–291 [PubMed: 22314066]
11. Gillespie BM, Chaboyer W, Erichsen-Andersson A, Hettiarachchi RM, and Kularatna S (2017) Economic case for intraoperative interventions to prevent surgical-site infection. British Journal of Surgery 104, e55–e64
12. Klevens RM, Edwards JR, Richards CL Jr., Horan TC, Gaynes RP, Pollock DA, and Cardo DM (2007) Estimating health care-associated infections and deaths in U.S. hospitals, 2002. Public Health Rep 122, 160–166 [PubMed: 17357358]
13. Glotzbecker MP, Riedel MD, Vitale MG, Matsumoto H, Roye DP, Erickson M, Flynn JM, and Saiman L (2013) What's the Evidence? Systematic Literature Review of Risk Factors and Preventive Strategies for Surgical Site Infection Following Pediatric Spine Surgery. Journal of Pediatric Orthopaedics 33, 479–487 [PubMed: 23752143]
14. Namba RS, Inacio MC, and Paxton EW (2013) Risk factors associated with deep surgical site infections after primary total knee arthroplasty: an analysis of 56,216 knees. J Bone Joint Surg Am 95, 775–782 [PubMed: 23636183]
15. Anderson DJ, Podgorny K, Berrios-Torres SI, Bratzler DW, Dellinger EP, Greene L, Nyquist AC, Saiman L, Yokoe DS, Maragakis LL, and Kaye KS (2014) Strategies to prevent surgical site

infections in acute care hospitals: 2014 update. Infect Control Hosp Epidemiol 35 Suppl 2, S66–88 [PubMed: 25376070]

16. Zimlichman E, Henderson D, Tamir O, Franz C, Song P, Yamin CK, Keohane C, Denham CR, and Bates DW (2013) Health care–associated infections: a meta-analysis of costs and financial impact on the US health care system. JAMA internal medicine 173, 2039–2046 [PubMed: 23999949]
17. Anderson DJ, Kaye KS, Chen LF, Schmader KE, Choi Y, Sloane R, and Sexton DJ (2009) Clinical and financial outcomes due to methicillin resistant Staphylococcus aureus surgical site infection: a multi-center matched outcomes study. PloS one 4, e8305 [PubMed: 20016850]
18. Bratzler DW, Houck PM, Surgical Infection Prevention Guidelines Writers, W., American Academy of Orthopaedic, S., American Association of Critical Care, N., American Association of Nurse, A., American College of, S., American College of Osteopathic, S., American Geriatrics, S., American Society of, A., American Society of, C., Rectal, S., American Society of Health-System, P., American Society of PeriAnesthesia, N., Ascension, H., Association of periOperative Registered, N., Association for Professionals in Infection, C., Epidemiology, Infectious Diseases Society of, A., Medical, L., Premier, Society for Healthcare Epidemiology of, A., Society of Thoracic, S., and Surgical Infection, S. (2004) Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. Clin Infect Dis 38, 1706–1715 [PubMed: 15227616]
19. Kirkland KB, Briggs JP, Trivette SL, Wilkinson WE, and Sexton DJ (1999) The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs. Infect Control Hosp Epidemiol 20, 725–730 [PubMed: 10580621]
20. Engemann JJ, Carmeli Y, Cosgrove SE, Fowler VG, Bronstein MZ, Trivette SL, Briggs JP, Sexton DJ, and Kaye KS (2003) Adverse clinical and economic outcomes attributable to methicillin resistance among patients with Staphylococcus aureus surgical site infection. Clinical infectious diseases 36, 592–598 [PubMed: 12594640]
21. Ban KA, Minei JP, Laronga C, Harbrecht BG, Jensen EH, Fry DE, Itani KM, Dellinger EP, Ko CY, and Duane TM (2017) American College of Surgeons and Surgical Infection Society: surgical site infection guidelines, 2016 update. Journal of the American College of Surgeons 224, 59–74 [PubMed: 27915053]
22. Allegranzi B, Zayed B, Bischoff P, Kubilay NZ, de Jonge S, de Vries F, Gomes SM, Gans S, Wallert ED, Wu X, Abbas M, Boermeester MA, Dellinger EP, Egger M, Gastmeier P, Guirao X, Ren J, Pittet D, Solomkin JS, and Group WHOGD (2016) New WHO recommendations on intraoperative and postoperative measures for surgical site infection prevention: an evidence-based global perspective. Lancet Infect Dis 16, e288–e303 [PubMed: 27816414]
23. Bratzler DW, Dellinger EP, Olsen KM, Perl TM, Auwaerter PG, Bolon MK, Fish DN, Napolitano LM, Sawyer RG, and Slain D (2013) Clinical practice guidelines for antimicrobial prophylaxis in surgery. Surgical infections 14, 73–156 [PubMed: 23461695]
24. Becattini S, Taur Y, and Pamer EG (2016) Antibiotic-Induced Changes in the Intestinal Microbiota and Disease. Trends Mol Med 22, 458–478 [PubMed: 27178527]
25. Jakobsson HE, Jernberg C, Andersson AF, Sjolund-Karlsson M, Jansson JK, and Engstrand L (2010) Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PloS one 5, e9836 [PubMed: 20352091]
26. Langdon A, Crook N, and Dantas G (2016) The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. Genome medicine 8, 1–16 [PubMed: 26750923]
27. Korpela K, Salonen A, Virta LJ, Kekkonen RA, Forslund K, Bork P, and De Vos WM (2016) Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. Nature communications 7, 1–8
28. Yassour M, Vatanen T, Siljander H, Hämäläinen A-M, Härkönen T, Ryhänen SJ, Franzosa EA, Vlamakis H, Huttenhower C, and Gevers D (2016) Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. Science translational medicine 8, 343ra381, 341–311
29. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, Challis C, Schretter CE, Rocha S, Gradinaru V, Chesselet M-F, Keshavarzian A, Shannon KM, Krajmalnik-Brown

R, Wittung-Stafshede P, Knight R, and Mazmanian SK (2014) Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell 167, 1469–1480.e1412

30. Brubaker SW, Bonham KS, Zanoni I, and Kagan JC (2015) Innate immune pattern recognition: a cell biological perspective. Annual review of immunology 33, 257–290
31. Janeway CA Jr., and Medzhitov R (2002) Innate immune recognition. Annu Rev Immunol 20, 197–216 [PubMed: 11861602]
32. Takeuchi O, and Akira S (2010) Pattern recognition receptors and inflammation. Cell 140, 805–820 [PubMed: 20303872]
33. Guo H, Callaway JB, and Ting JP (2015) Inflammasomes: mechanism of action, role in disease, and therapeutics. Nature medicine 21, 677–687
34. Martinon F, Mayor A, and Tschopp J (2009) The inflammasomes: guardians of the body. Annual review of immunology 27, 229–265
35. Schroder K, and Tschopp J (2010) The inflammasomes. Cell 140, 821–832 [PubMed: 20303873]
36. Kawai T, and Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34, 637–650 [PubMed: 21616434]
37. Dovi JV, Szpaderska AM, and DiPietro LA (2004) Neutrophil function in the healing wound: adding insult to injury? Thromb Haemost 92, 275–280 [PubMed: 15269822]
38. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, and Zychlinsky A (2004) Neutrophil extracellular traps kill bacteria. Science 303, 1532–1535 [PubMed: 15001782]
39. Velnar T, Bailey T, and Smrkolj V (2009) The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res 37, 1528–1542 [PubMed: 19930861]
40. Fenteany G, Janmey PA, and Stossel TP (2000) Signaling pathways and cell mechanics involved in wound closure by epithelial cell sheets. Curr. Biol 10, 831–838 [PubMed: 10899000]
41. Schafer M, and Werner S (2008) Cancer as an overhealing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol 9, 628–638 [PubMed: 18628784]
42. Martin P (1997) Wound healing--aiming for perfect skin regeneration. Science 276, 75–81 [PubMed: 9082989]
43. Diegelmann RF, and Evans MC (2004) Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci 9, 283–289 [PubMed: 14766366]
44. Agnese DM, Calvano JE, Hahm SJ, Coyle SM, Corbett SA, Calvano SE, and Lowry SF (2002) Human toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of gram-negative infections. The Journal of infectious diseases 186, 1522–1525 [PubMed: 12404174]
45. van de Vosse E, van Dissel JT, and Ottenhoff TH (2009) Genetic deficiencies of innate immune signalling in human infectious disease. The Lancet infectious diseases 9, 688–698 [PubMed: 19850227]
46. Kim YK, Shin J-S, and Nahm MH (2016) NOD-like receptors in infection, immunity, and diseases. Yonsei medical journal 57, 5–14 [PubMed: 26632377]
47. Drummond RA, and Lionakis MS (2016) Mechanistic insights into the role of C-type lectin receptor/CARD9 signaling in human antifungal immunity. Frontiers in cellular and infection microbiology 6, 1–11 [PubMed: 26870699]
48. Metruccio MM, Tam C, Evans DJ, Xie AL, Stern ME, and Fleiszig SM (2017) Contributions of MyD88-dependent receptors and CD11c-positive cells to corneal epithelial barrier function against Pseudomonas aeruginosa. Scientific reports 7, 1–14 [PubMed: 28127051]
49. Faure E, Mear JB, Faure K, Normand S, Couturier-Maillard A, Grandjean T, Balloy V, Ryffel B, Dessein R, Chignard M, Uyttenhove C, Guery B, Gosset P, Chamaillard M, and Kipnis E (2014) *Pseudomonas aeruginosa* type-3 secretion system dampens host defense by exploiting the NLRC4-coupled inflammasome. Am J Respir Crit Care Med 189, 799–811 [PubMed: 24555512]
50. Sharma P, Guha S, Garg P, and Roy S (2018) Differential expression of antimicrobial peptides in corneal infection and regulation of antimicrobial peptides and reactive oxygen species by type III secretion system of Pseudomonas aeruginosa. Pathogens and disease 76, 1–9

51. Lu Z, Casalino-Matsuda SM, Nair A, Buchbinder A, Budinger GS, Sporn PH, and Gates KL (2018) A role for heat shock factor 1 in hypercapnia-induced inhibition of inflammatory cytokine expression. The FASEB Journal 32, 3614–3622 [PubMed: 29405096]
52. Wang N, Gates KL, Trejo H, Favoreto S, Schleimer RP, Sznajder JI, Beitel GJ, and Sporn PH (2010) Elevated CO2 selectively inhibits interleukin-6 and tumor necrosis factor expression and decreases phagocytosis in the macrophage. The FASEB Journal 24, 2178–2190 [PubMed: 20181940]
53. Schröder NW, Morath S, Alexander C, Hamann L, Hartung T, Zähringer U, Göbel UB, Weber JR, and Schumann RR (2003) Lipoteichoic acid (LTA) of Streptococcus pneumoniaeand Staphylococcus aureus activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. Journal of Biological Chemistry 278, 15587–15594
54. Takeuchi O, Hoshino K, and Akira S (2000) Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. The Journal of Immunology 165, 5392–5396 [PubMed: 11067888]
55. Miller LS, O'connell RM, Gutierrez MA, Pietras EM, Shahangian A, Gross CE, Thirumala A, Cheung AL, Cheng G, and Modlin RL (2006) MyD88 mediates neutrophil recruitment initiated by IL-1R but not TLR2 activation in immunity against Staphylococcus aureus. Immunity 24, 79–91 [PubMed: 16413925]
56. Clark SC, and Kamen R (1987) The human hematopoietic colony-stimulating factors. Science 236, 1229–1237 [PubMed: 3296190]
57. Wakefield PE, James WD, Samlaska CP, and Meltzer MS (1990) Colony-stimulating factors. Journal of the American Academy of Dermatology 23, 903–912 [PubMed: 2254475]
58. Bussolino F, Wang JM, Defilippi P, Turrini F, Sanavio F, Edgell C-J, Aglietta M, Arese P, and Mantovani A (1989) Granulocyte-and granulocyte–macrophage-colony stimulating factors induce human endothelial cells to migrate and proliferate. Nature 337, 471–473 [PubMed: 2464767]
59. Bhattacharya P, Budnick I, Singh M, Thiruppathi M, Alharshawi K, Elshabrawy H, Holterman MJ, and Prabhakar BS (2015) Dual role of GM-CSF as a pro-inflammatory and a regulatory cytokine: implications for immune therapy. Journal of Interferon & Cytokine Research 35, 585–599 [PubMed: 25803788]
60. Mehta HM, Malandra M, and Corey SJ (2015) G-CSF and GM-CSF in Neutropenia. The Journal of Immunology 195, 1341–1349 [PubMed: 26254266]
61. Kim MH, Liu W, Borjesson DL, Curry FR, Miller LS, Cheung AL, Liu FT, Isseroff RR, and Simon SI (2008) Dynamics of neutrophil infiltration during cutaneous wound healing and infection using fluorescence imaging. The Journal of investigative dermatology 128, 1812–1820 [PubMed: 18185533]
62. Kienle K, and Lämmermann T (2016) Neutrophil swarming: an essential process of the neutrophil tissue response. Immunological reviews 273, 76–93 [PubMed: 27558329]
63. De Oliveira S, Rosowski EE, and Huttenlocher A (2016) Neutrophil migration in infection and wound repair: going forward in reverse. Nature Reviews Immunology 16, 378–391
64. Bowie AG, and Unterholzner L (2008) Viral evasion and subversion of pattern-recognition receptor signalling. Nature Reviews Immunology 8, 911–922
65. Albrecht C, Boutrot F, Segonzac C, Schwessinger B, Gimenez-Ibanez S, Chinchilla D, Rathjen JP, de Vries SC, and Zipfel C (2012) Brassinosteroids inhibit pathogen-associated molecular pattern–triggered immune signaling independent of the receptor kinase BAK1. Proceedings of the National Academy of Sciences 109, 303–308
66. Abramovitch RB, Anderson JC, and Martin GB (2006) Bacterial elicitation and evasion of plant innate immunity. Nature Reviews Molecular Cell Biology 7, 601–611 [PubMed: 16936700]
67. Trdá L, Boutrot F, Claverie J, Brulé D, Dorey S, and Poinssot B (2015) Perception of pathogenic or beneficial bacteria and their evasion of host immunity: pattern recognition receptors in the frontline. Frontiers in plant science 6, 1–11 [PubMed: 25653664]
68. Taxman DJ, Huang MT, and Ting JP (2010) Inflammasome inhibition as a pathogenic stealth mechanism. Cell Host Microbe 8, 7–11 [PubMed: 20638636]

69. Mangram AJ, Horan TC, Pearson ML, Silver LC, and Jarvis WR (1999) Guideline for Prevention of Surgical Site Infection, 1999. Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. Am J Infect Control 27, 97–132; quiz 133–134; discussion 196 [PubMed: 10196487]
70. Berríos-Torres SI, Umscheid CA, Bratzler DW, Leas B, Stone EC, Kelz RR, Reinke CE, Morgan S, Solomkin JS, and Mazuski JE (2017) Centers for Disease Control and Prevention guideline for the prevention of surgical site infection, 2017. JAMA surgery 152, 784–791 [PubMed: 28467526]
71. Goldufsky J, Wood SJ, Jayaraman V, Majdobeh O, Chen L, Qin S, Zhang C, DiPietro LA, and Shafikhani SH (2015) *Pseudomonas aeruginosa* uses T3SS to inhibit diabetic wound healing. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 23, 557–564
72. Tosh PK, Disbot M, Duffy JM, Boom ML, Heseltine G, Srinivasan A, Gould CV, and Berrios-Torres SI (2011) Outbreak of Pseudomonas aeruginosa surgical site infections after arthroscopic procedures: Texas, 2009. Infect Control Hosp Epidemiol 32, 1179–1186 [PubMed: 22080656]
73. Brouqui P, Rousseau M, Stein A, Drancourt M, and Raoult D (1995) Treatment of Pseudomonas aeruginosa-infected orthopedic prostheses with ceftazidime-ciprofloxacin antibiotic combination. Antimicrobial agents and chemotherapy 39, 2423–2425 [PubMed: 8585720]
74. Arciola CR, An Y, Campoccia D, Donati M, and Montanaro L (2005) Etiology of implant orthopedic infections: a survey on 1027 clinical isolates. The International journal of artificial organs 28, 1091–1100 [PubMed: 16353115]
75. Redel H, Gao Z, Li H, Alekseyenko AV, Zhou Y, Perez-Perez GI, Weinstock G, Sodergren E, and Blaser MJ (2013) Quantitation and composition of cutaneous microbiota in diabetic and nondiabetic men. The Journal of infectious diseases 207, 1105–1114 [PubMed: 23300163]
76. Panaro M, and Mitolo V (1999) Cellular responses to FMLP challenging: a mini-review. Immunopharmacology and immunotoxicology 21, 397–419 [PubMed: 10466071]
77. Balazovich KJ, Suchard SJ, Remick DG, and Boxer L (1996) Tumor necrosis factor-alpha and FMLP receptors are functionally linked during FMLP-stimulated activation of adherent human neutrophils. Blood 88, 690–696 [PubMed: 8695817]
78. Derian CK, Santulli RJ, Rao PE, Solomon HF, and Barrett JA (1995) Inhibition of chemotactic peptide-induced neutrophil adhesion to vascular endothelium by cAMP modulators. The Journal of Immunology 154, 308–317 [PubMed: 7995950]
79. Madianos P, Bobetsis Y, and Kinane D (2005) Generation of inflammatory stimuli: how bacteria set up inflammatory responses in the gingiva. Journal of Clinical Periodontology 32, 57–71 [PubMed: 16128830]
80. Sabroe I, Prince LR, Jones EC, Horsburgh MJ, Foster SJ, Vogel SN, Dower SK, and Whyte MK (2003) Selective roles for Toll-like receptor (TLR)2 and TLR4 in the regulation of neutrophil activation and life span. Journal of immunology 170, 5268–5275
81. Liu M, Chen K, Yoshimura T, Liu Y, Gong W, Wang A, Gao J-L, Murphy PM, and Wang JM (2012) Formylpeptide receptors are critical for rapid neutrophil mobilization in host defense against Listeria monocytogenes. Scientific reports 2, 1–7
82. Bhavsar I, Miller CS, and Al-Sabbagh M (2015) Macrophage inflammatory protein-1 alpha (MIP-1 alpha)/CCL3: as a biomarker. General methods in biomarker research and their applications, 223–249
83. Chou RC, Kim ND, Sadik CD, Seung E, Lan Y, Byrne MH, Haribabu B, Iwakura Y, and Luster AD (2010) Lipid-cytokine-chemokine cascade drives neutrophil recruitment in a murine model of inflammatory arthritis. Immunity 33, 266–278 [PubMed: 20727790]
84. Luster AD, Alon R, and von Andrian UH (2005) Immune cell migration in inflammation: present and future therapeutic targets. Nature immunology 6, 1182–1190 [PubMed: 16369557]
85. He HQ, Liao D, Wang ZG, Wang ZL, Zhou HC, Wang MW, and Ye RD (2013) Functional characterization of three mouse formyl peptide receptors. Molecular pharmacology 83, 389–398 [PubMed: 23160941]
86. Zibert A, Balzer S, Souquet M, Quang TH, Paris-Scholz C, Roskrow M, and Dilloo D (2004) CCL3/MIP-1 α Is a Potent Immunostimulator When Coexpressed with Interleukin-2 or

Granulocyte-Macrophage Colony-Stimulating Factor in a Leukemia/Lymphoma Vaccine. Human gene therapy 15, 21–34 [PubMed: 14965375]

87. Kayagaki N, Warming S, Lamkanfi M, Walle LV, Louie S, Dong J, Newton K, Qu Y, Liu J, and Heldens S (2011) Non-canonical inflammasome activation targets caspase-11. Nature 479, 117–121 [PubMed: 22002608]
88. Roy R, Zayas J, Mohamed MF, Aboonabi A, Delgado K, Wallace J, Bayat M, Kuzel TM, Reiser J, and Shafikhani SH (2021) IL-10 Dysregulation Underlies Chemokine Insufficiency, Delayed Macrophage Response, and Impaired Healing in Diabetic Wound. Journal of Investigative Dermatology
89. Wood S, Jayaraman V, Huelsmann EJ, Bonish B, Burgad D, Sivaramakrishnan G, Qin S, Dipietro LA, Zloza A, Zhang C, and Shafikhani SH (2014) Pro-inflammatory chemokine CCL2 (MCP-1) promotes healing in diabetic wounds by restoring the macrophage response. PloS one 9, e91574 [PubMed: 24618995]
90. Roy R, Zayas J, Mohamed MF, Aboonabi A, Delgado K, Wallace J, Bayat M, Kuzel TM, Reiser J, and Shafikhani SH (2021) IL-10 Dysregulation Underlies Chemokine Insufficiency, Delayed Macrophage Response, and Impaired Healing in Diabetic Wound. Journal of Investigative Dermatology In press
91. Shafikhani SH, and Engel J (2006) *Pseudomonas aeruginosa* type III-secreted toxin ExoT inhibits host-cell division by targeting cytokinesis at multiple steps. Proceedings of the National Academy of Sciences of the United States of America 103, 15605–15610 [PubMed: 17030800]
92. Wood SJ, Goldufsky J, and Shafikhani SH (2015) *Pseudomonas aeruginosa* ExoT Induces Atypical Anoikis Apoptosis in Target Host Cells by Transforming Crk Adaptor Protein into a Cytotoxin. PLoS pathogens 11, e1004934 [PubMed: 26020630]
93. Wood SJ, Goldufsky JW, Bello D, Masood S, and Shafikhani SH (2015) *Pseudomonas aeruginosa* ExoT induces mitochondrial apoptosis in target host cells in a manner that depends on its GTPase-activating protein (GAP) domain activity. Journal of Biological Chemistry 290, 29063–29073
94. Kroin JS, Li J, Shafikhani S, Gupta KH, Moric M, and Buvanendran A (2018) Local vancomycin effectively reduces surgical site infection at implant site in rodents. Regional Anesthesia & Pain Medicine 43, 795–804 [PubMed: 29905629]
95. Hamilton JL, Mohamed MF, Witt BR, Wimmer MA, and Shafikhani SH (2021) Therapeutic assessment of N-formyl-methionyl-leucyl-phenylalanine (fMLP) in reducing periprosthetic joint infection. Eur Cell Mater 41, 122–138
96. Hsu C-Y, Shu J-C, Lin M-H, Chong K-Y, Chen C-C, Wen S-M, Hsieh Y-T, and Liao W-T (2015) High glucose concentration promotes vancomycin-enhanced biofilm formation of vancomycin-non-susceptible Staphylococcus aureus in diabetic mice. PloS one 10, e0134852 [PubMed: 26244880]
97. Teneback CC, Scanlon TC, Wargo MJ, Bement JL, Griswold KE, and Leclair LW (2013) Bioengineered lysozyme reduces bacterial burden and inflammation in a murine model of mucoid Pseudomonas aeruginosa lung infection. Antimicrob Agents Chemother 57, 5559–5564 [PubMed: 23979752]
98. Gupta KH, Goldufsky JW, Wood SJ, Tardi NJ, Moorthy GS, Gilbert DZ, Zayas JP, Hahm E, Altintas MM, Reiser J, and Shafikhani SH (2017) Apoptosis and Compensatory Proliferation Signaling Are Coupled by CrkI-Containing Microvesicles. Dev Cell 41, 674–684 e675 [PubMed: 28633020]
99. Brubaker AL, Rendon JL, Ramirez L, Choudhry MA, and Kovacs EJ (2013) Reduced neutrophil chemotaxis and infiltration contributes to delayed resolution of cutaneous wound infection with advanced age. The Journal of Immunology 190, 1746–1757 [PubMed: 23319733]
100. Christou NV, Mannick JA, West MA, and Kasper DL (1987) Lymphocyte-macrophage interactions in the response to surgical infections. Archives of Surgery 122, 239–251 [PubMed: 3492987]
101. Wagner C, Iking-Konert C, Hug F, Stegmaier S, Heppert V, Wentzensen A, and Hänsch G (2006) Cellular inflammatory response to persistent localized Staphylococcus aureus infection: phenotypical and functional characterization of polymorphonuclear neutrophils (PMN). Clinical & Experimental Immunology 143, 70–77 [PubMed: 16367936]

102. Daley JM, Thomay AA, Connolly MD, Reichner JS, and Albina JE (2008) Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. Journal of leukocyte biology 83, 64–70 [PubMed: 17884993]
103. Klebanoff SJ (2005) Myeloperoxidase: friend and foe. Journal of leukocyte biology 77, 598–625 [PubMed: 15689384]
104. Hodson M, Gallagher C, and Govan J (2002) A randomised clinical trial of nebulised tobramycin or colistin in cystic fibrosis. European Respiratory Journal 20, 658–664
105. Shawar RM, MacLeod DL, Garber RL, Burns JL, Stapp JR, Clausen CR, and Tanaka S (1999) Activities of tobramycin and six other antibiotics against Pseudomonas aeruginosa isolates from patients with cystic fibrosis. Antimicrobial agents and chemotherapy 43, 2877–2880 [PubMed: 10582875]
106. Bothra M, Lodha R, and Kabra SK (2012) Tobramycin for the treatment of bacterial pneumonia in children. Expert opinion on pharmacotherapy 13, 565–571 [PubMed: 22292783]
107. Wood CA, Norton DR, Kohlhepp SJ, Kohnen PW, Porter GA, Houghton DC, Brummett RE, Bennett WM, and Gilbert DN (1988) The influence of tobramycin dosage regimens on nephrotoxicity, ototoxicity, and antibacterial efficacy in a rat model of subcutaneous abscess. Journal of Infectious Diseases 158, 13–22
108. Hoff GE, Schiøtz PO, and Paulsen J (1974) Tobramycin treatment of Pseudomonas aeruginosa infections in cystic fibrosis. Scandinavian journal of infectious diseases 6, 333–337 [PubMed: 4217466]
109. Avent M, Rogers B, Cheng A, and Paterson D (2011) Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity. Internal medicine journal 41, 441–449 [PubMed: 21309997]
110. Bjarnsholt T, Kirketerp-Moller K, Jensen PO, Madsen KG, Phipps R, Krogfelt K, Hoiby N, and Givskov M (2008) Why chronic wounds will not heal: a novel hypothesis. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 16, 2–10
111. Menke NB, Ward KR, Witten TM, Bonchev DG, and Diegelmann RF (2007) Impaired wound healing. Clin Dermatol 25, 19–25 [PubMed: 17276197]
112. Blakytny R, and Jude E (2006) The molecular biology of chronic wounds and delayed healing in diabetes. Diabet Med 23, 594–608 [PubMed: 16759300]
113. Gaynes RP, Culver DH, Horan TC, Edwards JR, Richards C, Tolson JS, and System NNIS (2001) Surgical site infection (SSI) rates in the United States, 1992–1998: the National Nosocomial Infections Surveillance System basic SSI risk index. Clinical Infectious Diseases 33, S69–S77 [PubMed: 11486302]
114. Smyth E, and Emmerson A (2000) Surgical site infection surveillance. Journal of Hospital Infection 45, 173–184
115. Koh TJ, and DiPietro LA (2011) Inflammation and wound healing: the role of the macrophage. Expert reviews in molecular medicine 13, 1–11
116. Rivas-Santiago B, Serrano CJ, and Enciso-Moreno JA (2009) Susceptibility to infectious diseases based on antimicrobial peptide production. Infection and immunity 77, 4690–4695 [PubMed: 19703980]
117. Singer AJ, and Clark RA (1999) Cutaneous wound healing. N Engl J Med 341, 738–746 [PubMed: 10471461]
118. Jones SG, Edwards R, and Thomas DW (2004) Inflammation and wound healing: the role of bacteria in the immuno-regulation of wound healing. Int J Low Extrem Wounds 3, 201–208 [PubMed: 15866816]
119. De Smet K, and Contreras R (2005) Human antimicrobial peptides: defensins, cathelicidins and histatins. Biotechnol Lett 27, 1337–1347 [PubMed: 16215847]
120. Saunders KT, Slaughter GT, Mercer L, and Gerkin R (2018) Antibiotic Prophylaxis in Surgical Sterilization: Following the Recommendations [29g]. Obstetrics & Gynecology 131, 82S–83S
121. Hughes WT, Armstrong D, Bodey GP, Bow EJ, Brown AE, Calandra T, Feld R, Pizzo PA, Rolston KV, Shenep JL, and Young LS (2002) 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Infect Dis 34, 730–751 [PubMed: 11850858]

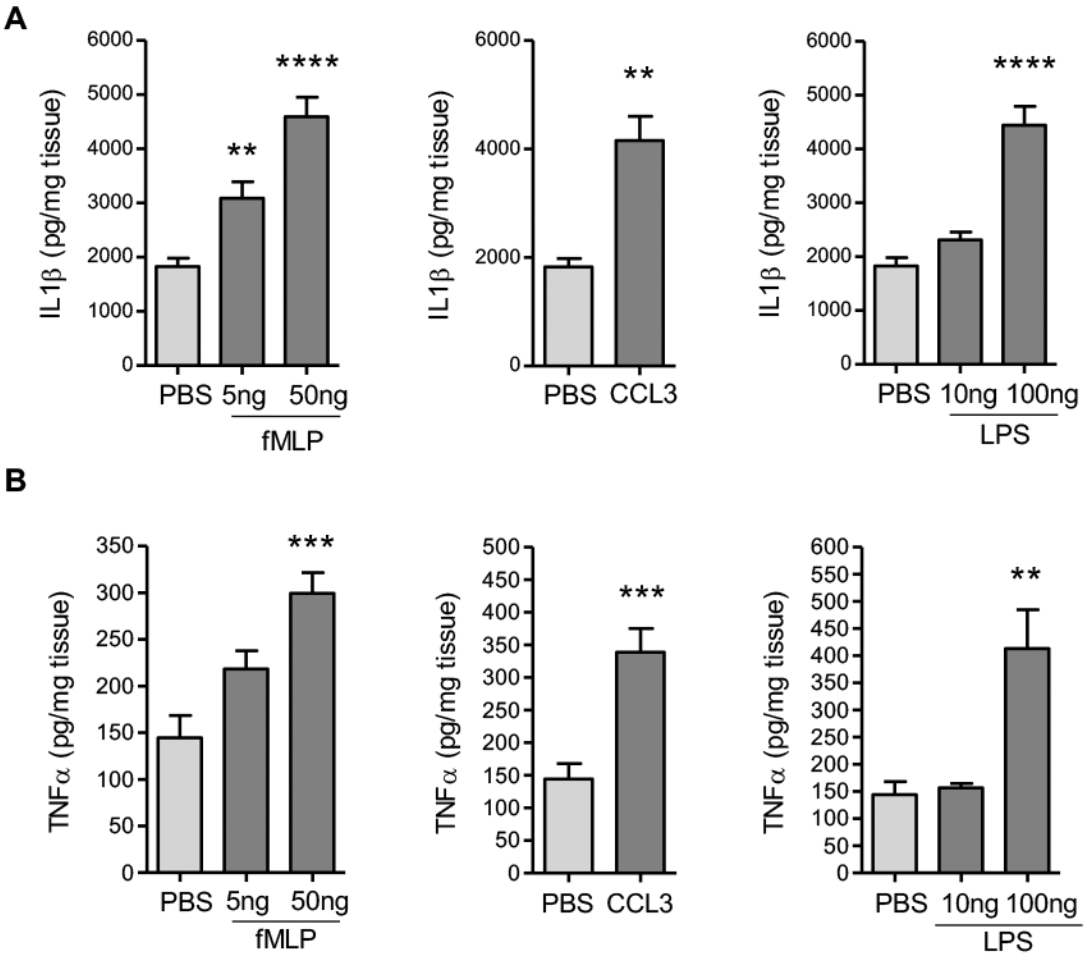

Figure 1. Immunomodulators increase proinflammatory cytokines production in wounds.

Wounds on C57B mice were topically treated with the indicated immunomodulators (fMLP at 5ng or 50ng/wound; CCL3 at 1μg/wound; and LPS at 10ng or 100ng/wound) or PBS control after wounding. Wounds were then infected with PA103 *P. aeruginosa* strain (at 10^6 bacteria/wound). 24 hours after infection, the levels of IL-1β (A) and TNF-α (B) in wounds were determined by ELISA after normalization with tissue weight. The corresponding data are shown as Mean ± SEM. (7 mice/group were used in this study except for fMLP at 5ng/wound and LPS at 10ng/wound where n=3 mice/groups. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

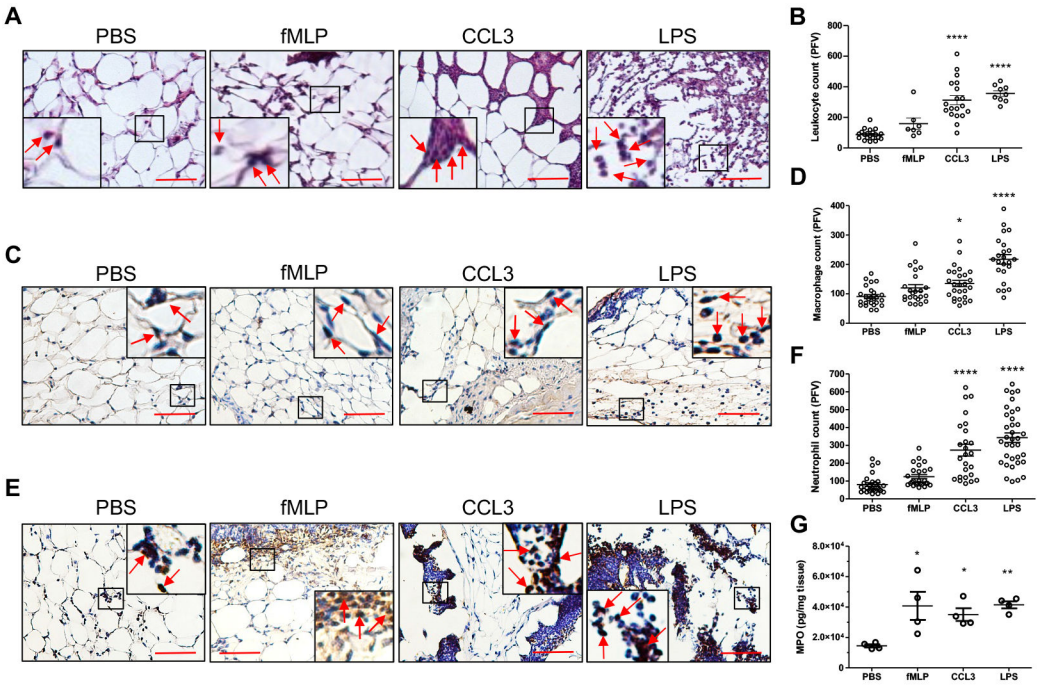

Figure 2. Immunomodulators enhance leukocytes influx and neutrophil activation in infected wound.

C57BL/6 wounds were treated with PBS or received topical treatments with fMLP, CCL3, and LPS, (at 50ng, 1μg, and 100ng per wound, respectively), before infection with 10^6 PA103. 24 hours after infection, wounds were examined for their leukocytes' contents by histological analysis, using H&E staining (A & B); for their macrophage contents using anti-CD68 antibody (C & D); and for their neutrophil contents using anti-Ly6G (E & F). Representative regions from underneath the wounds extending in the dermis are shown in (A, C, & E), and the corresponding data are plotted as Mean ± SEM and shown in (B, D, & F). (Inserts are the magnified regions indicated by smaller rectangles within the images. Red scale bar = 50μm). (G) The aforementioned wounds were assessed for their activated neutrophil contents by evaluating their MPO contents by ELISA. (N≥3 mice/group, ≥9 random fields/wound/mouse. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

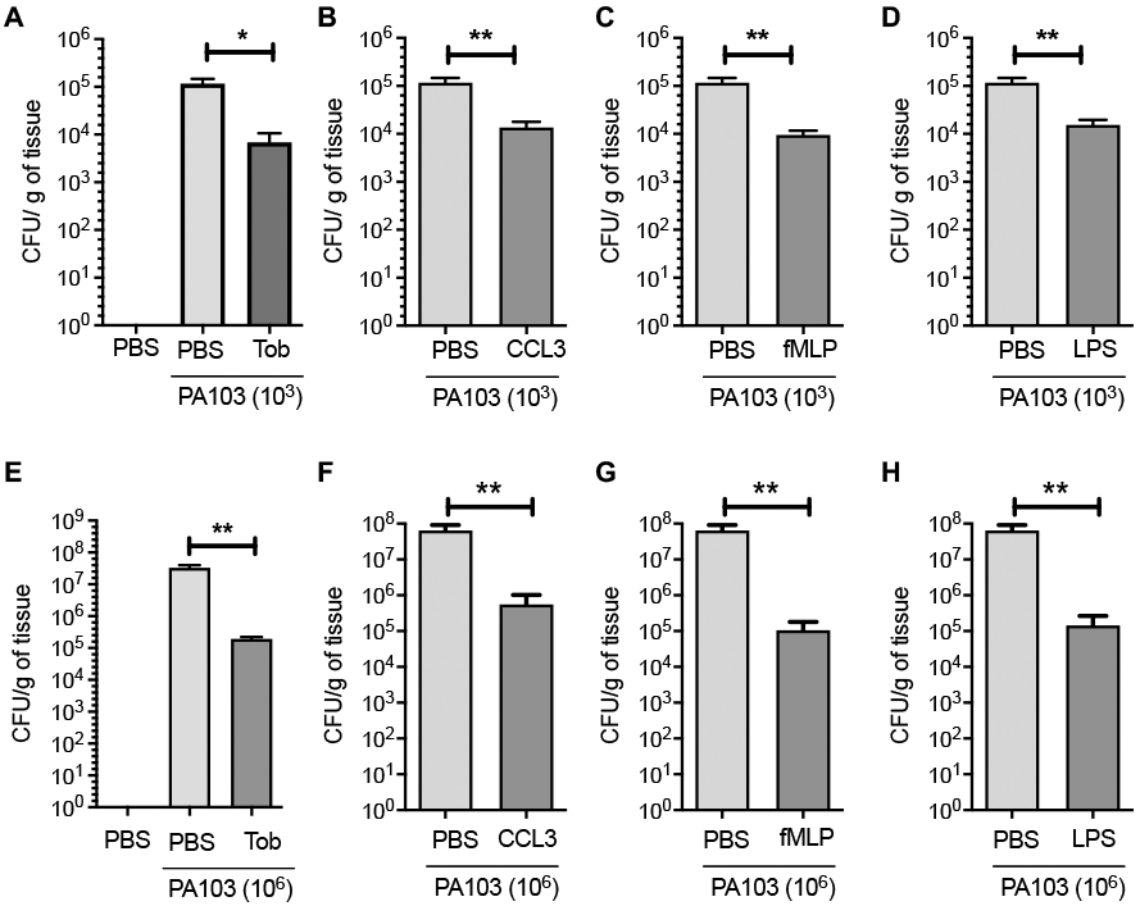

Figure 3. Immunomodulators enhance infection control in wound.

(A and E) C57BL/6 mice received systemic Tobramycin (Tob) or PBS or by i.p. injection 1h before surgical wounding. They were then infected with PA103 at 10^3 (A) or 10^6 (E) bacteria/wound. Wounds were collected at 24h after infection and assessed for their bacterial contents by bacterial colony forming unit determination (CFU). (B-D and F-H) C57BL/6 wounds were infected with either 10^3 (B-D) or 10^6 (F-H) PA103 bacteria per wound after treatment with fMLP, CCL3, and LPS immunomodulators. Wounds were then collected 24h after treatment, and infection was assessed for their bacterial contents by CFU determination. Data were normalized with their corresponding wound tissue weight. (These experiments were performed twice, each time with N=4 mice/group; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

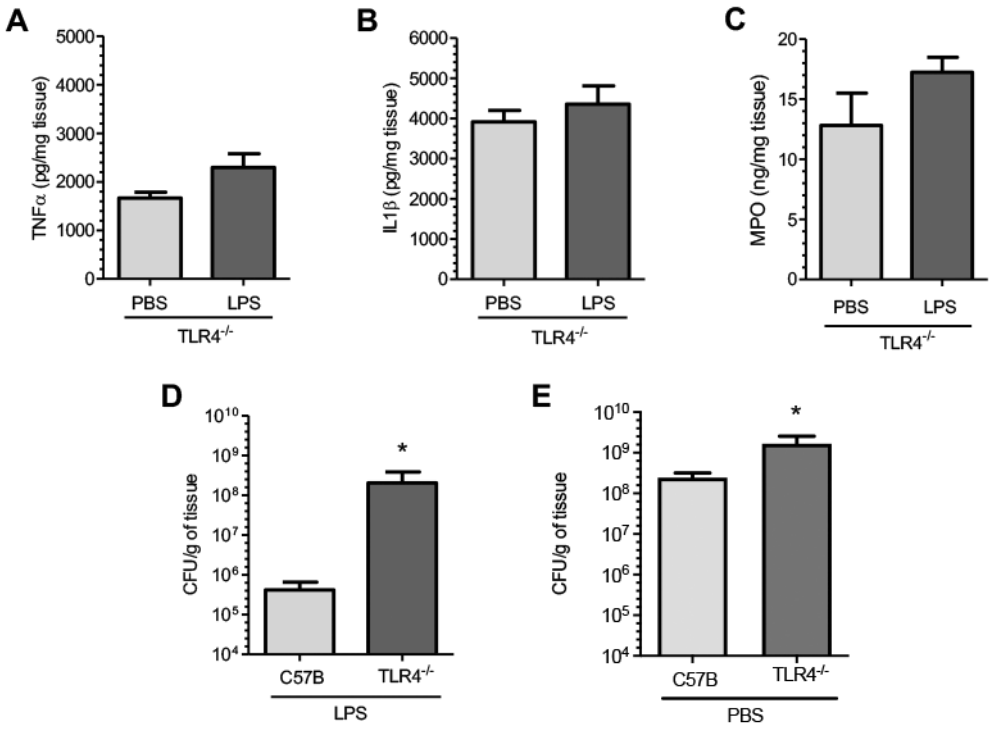

Figure 4. LPS-induced enhancement in infection control in wound is dependent on TLR4 receptor signaling.

(A-C) Wounds in TLR4$^{-/-}$ mice were treated with PBS or received LPS (100ng/wound) topical treatment before infection with 10^6 PA103. 24 hours after infection, wounds were examined for TNF-α (A), for IL-1β (B), for activated neutrophils, using MPO (C), all by ELISA. (D) Wounds in C57BL/6 and TLR4$^{-/-}$ mice were treated with PBS or received LPS (100ng/wound) topical treatment before infection with 10^6 PA103. 24 hours after infection, wounds were assessed for their bacterial counts by CFU determination. The corresponding data are plotted as Mean ± SEM. (N=4 mice/group; *p<0.05).

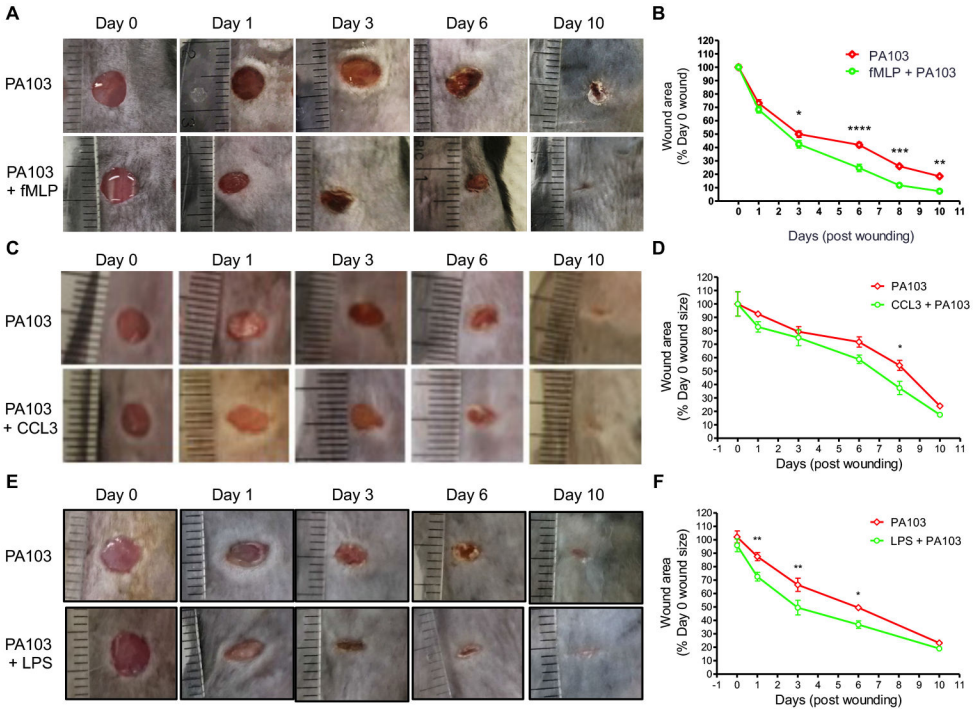

Figure 5. Immunomodulators do not adversely affect healing in infected wounds.

(A-F) Wounds in C57BL/6 mice were treated with fMLP (50ng), CCL3 (1μg), or LPS (100ng) per wound prior to infection with 10^6 PA103. Wound healing was assessed by digital photography at day 0 (at the time of wounding and treatment) and at days 1, 3, 6, and 10 post-infection. Representative wound images are shown in (A, C, & E) and the corresponding data are plotted as the Mean ± SEM and shown in (B, D, & F). (N≥4 mice/group; *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001).

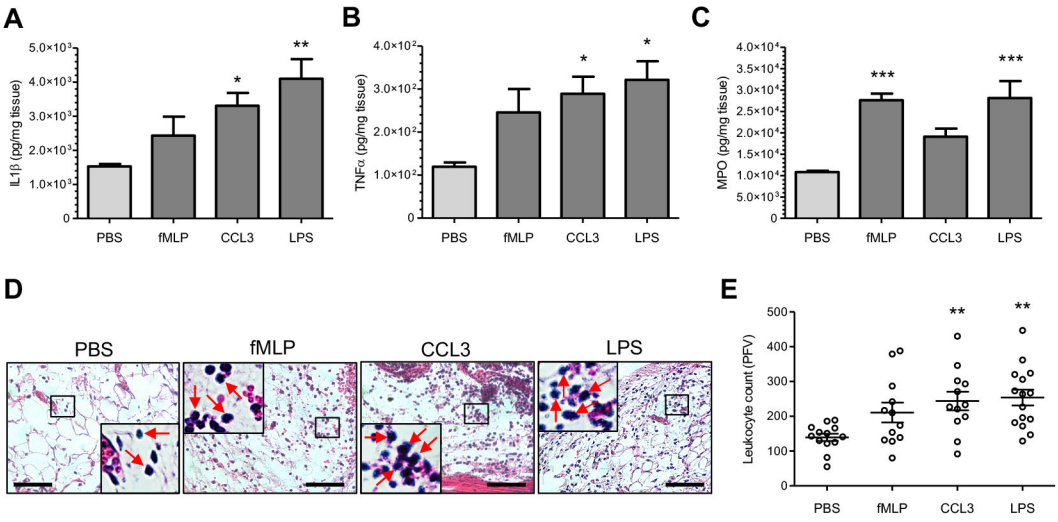

Figure 6. Immunomodulators enhance proinflammatory responses in uninfected wound.

Wounds in C57BL/6 were treated with PBS or received fMLP, CCL3, or LPS (at 50ng, 1μg, or 100ng per wound, respectively). 24 hours after treatment, wounds were analyzed for their IL-1β (A), for TNF-α (B), for their MPO contents (C), all by ELISA. (D-E) The aforementioned wounds were assessed for their leukocytes' contents by histological analysis, using H&E staining. Representative regions from underneath the wounds extending in the dermis are shown at indicated magnification in (D) and the corresponding data are plotted as Mean ± SEM and shown in (E). Black scale bar = 50μm). (Inserts are the magnified regions indicated by smaller rectangles within the images). (N=4 mice/group; *p<0.05, **p<0.01, ***p<0.001).

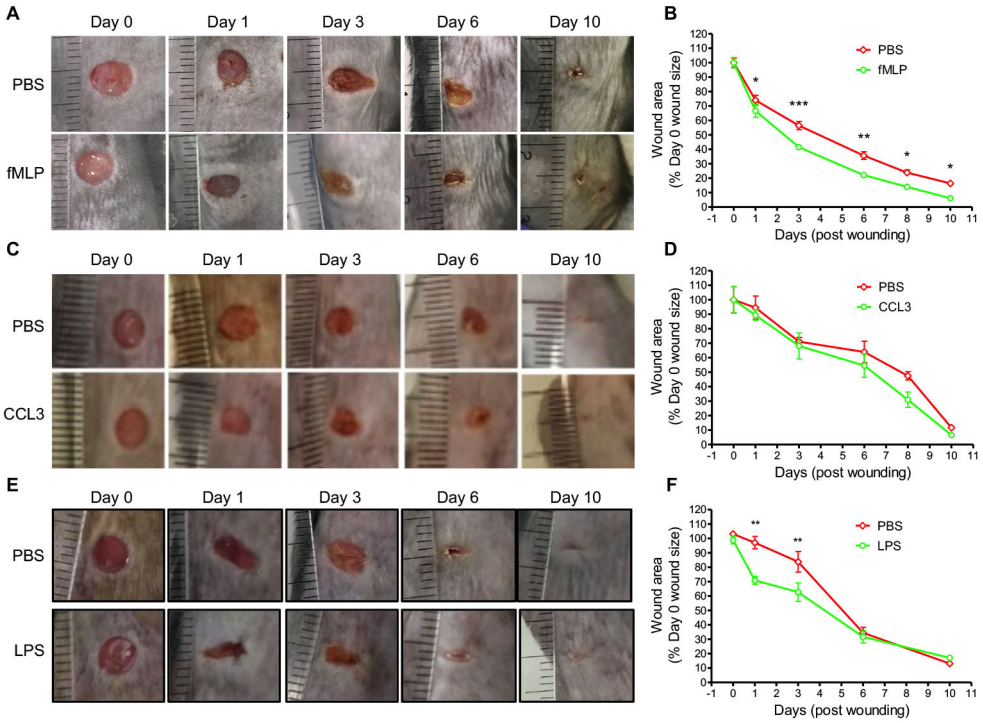

Figure 7. Treatment with immunomodulators do not adversely affect healing in uninfected wounds.

Wounds in C57BL/6 were treated with PBS or received fMLP, CCL3, or LPS at 50ng, 1μg, and 100ng per wound respectively. Wound healing was assessed by digital photography at day 0 (at the time of wounding at treatment), and at days 1, 3, 6, and 10 post-wounding and treatment. Representative wound images are shown in (A, C, & E) and the corresponding data are plotted as the Mean ± SEM and shown in (B, D, & F). (N≥4 mice/group; *p<0.05, **p<0.01, ***p<0.001).