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Award Number: W81XWH-10-1-0872

TITLE: Inhibition of Orthopaedic Implant Infections by Immunomodulatory Effects of Host Defense Peptides

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REPORT DATE: October 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE October 2013		2. REPORT TYPE Annual		3. DATES COVERED 15 September 2012 - 14 September 2013	
4. TITLE AND SUBTITLE Inhibition of Orthopaedic Implant Infections by Immunomodulatory Effects of Host Defense Peptides				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-10-1-0872	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Edward Greenfield, Ph.D. E-Mail: emg3@cwru.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Case Western Reserve University Cleveland OH 44106-1712				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Host defense peptides represent a promising new approach to inhibit infection. The anti-infective actions of these peptides are primarily due to their immunomodulatory effects. Since they regulate multiple aspects of the mammalian immune system, host defense peptides are also less likely to induce bacterial resistance than are traditional antibiotics. The local delivery of anti-bacterial agents allows for both high local concentrations to increase efficacy and low systemic levels to reduce toxicity. A promising strategy for the local delivery of anti-bacterial agents is to bind them directly to the surfaces of orthopaedic implants. The key research accomplishments in the reporting period are: 1. The soluble host defense peptide increases production of MCP-1 but decreases the ability of LPS to induce TNFα or MCP-1. 2. The soluble host defense peptide reduces effects of S. aureus on orthopaedic implant osseointegration. 3. Development of a quantitative and reproducible murine model of A. baumannii orthopaedic implant infection. The host defense peptides have the potential to substantially reduce infections of fractures sustained on the battlefield and in civilian settings. If the synthetic peptide reduces infections in the studies proposed in this application, more extensive pre-clinical testing would precisely determine its potential benefits and risks and determine whether the peptide is a high priority for human trials.					
15. SUBJECT TERMS Orthopaedic infections, Host Defense Peptides, Murine model, Staphylococcus aureus, Acinetobacter baumannii					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			USAMRMC
			UU	8	19b. TELEPHONE NUMBER (include area code)

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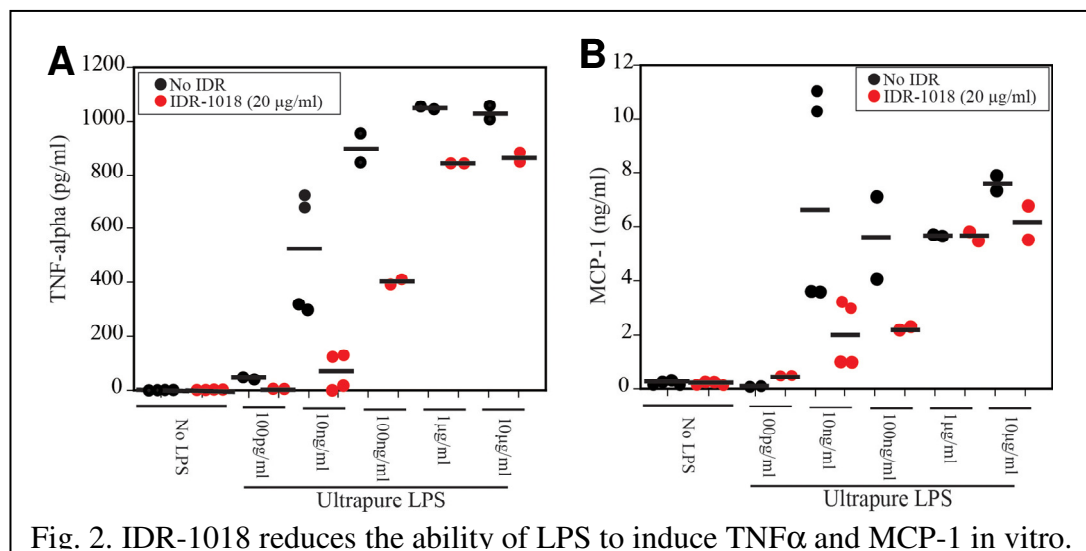
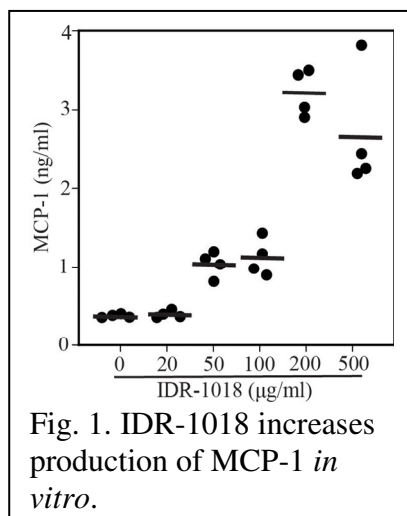
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INTRODUCTION:

Host defense peptides represent a promising new approach to inhibit infection. The anti-infective actions of these peptides are primarily due to their immunomodulatory effects. Since they regulate multiple aspects of the mammalian immune system, host defense peptides are also less likely to induce bacterial resistance than are traditional antibiotics. The purpose of this project is to assess whether host defense peptides are a promising strategy for treating infected orthopaedic implants. The scope of the project is to measure effects of host defense peptides on macrophages *in vitro* and on implants infected with *Staph. aureus* or *Acinetobacter baumannii* in our murine model of implant osseointegration.

BODY:

Progress in this year has primarily been in three areas of research. The first primary area of progress is the *in vitro* effects of the host defense peptides. We have found that the host defense peptide IDR-1018 stimulates production of MCP-1 (Fig. 1). IDR-1018 also inhibits the ability of LPS to stimulate production of TNF α and MCP-1 (Fig. 2). Thus, the effects of IDR-1018 are similar to what has been reported in the literature [1] but the effect in Figure 2B has not been shown previously. The results in Figure 2B suggest that anti-infective effects of IDR-1018 are likely more complex than the previous understanding which was that the anti-infective effects are primarily due to stimulation of production of chemokines such as MCP-1.



The second primary area of progress is the *in vivo* effects of IDR-1018 on infection with *S. aureus*. IDR-1018 reduced the rate of gross integration failures from 60% (6 of 10 mice) to 33% (3 of 9 mice). IDR-1018 also modestly increased osseointegration as assessed by biomechanical pull-out testing (Fig. 3A-C). IDR-1018 also reduced bacterial burden as assessed by bioluminescence (Fig. 3D) and by measuring colony forming units (CFUs) and *Lux* gene copies in the surrounding bone (Fig. 3E-F). In contrast, bacteria adherent to the implants were not affected (Fig. 3E-F). Those results are likely due to the adherent bacteria forming a biofilm which protects them from the host immune system. Those results also suggest that IDR-1018 may be substantially more effective when applied in combination with removal of the infected implant hardware, which is the most likely clinical scenario.

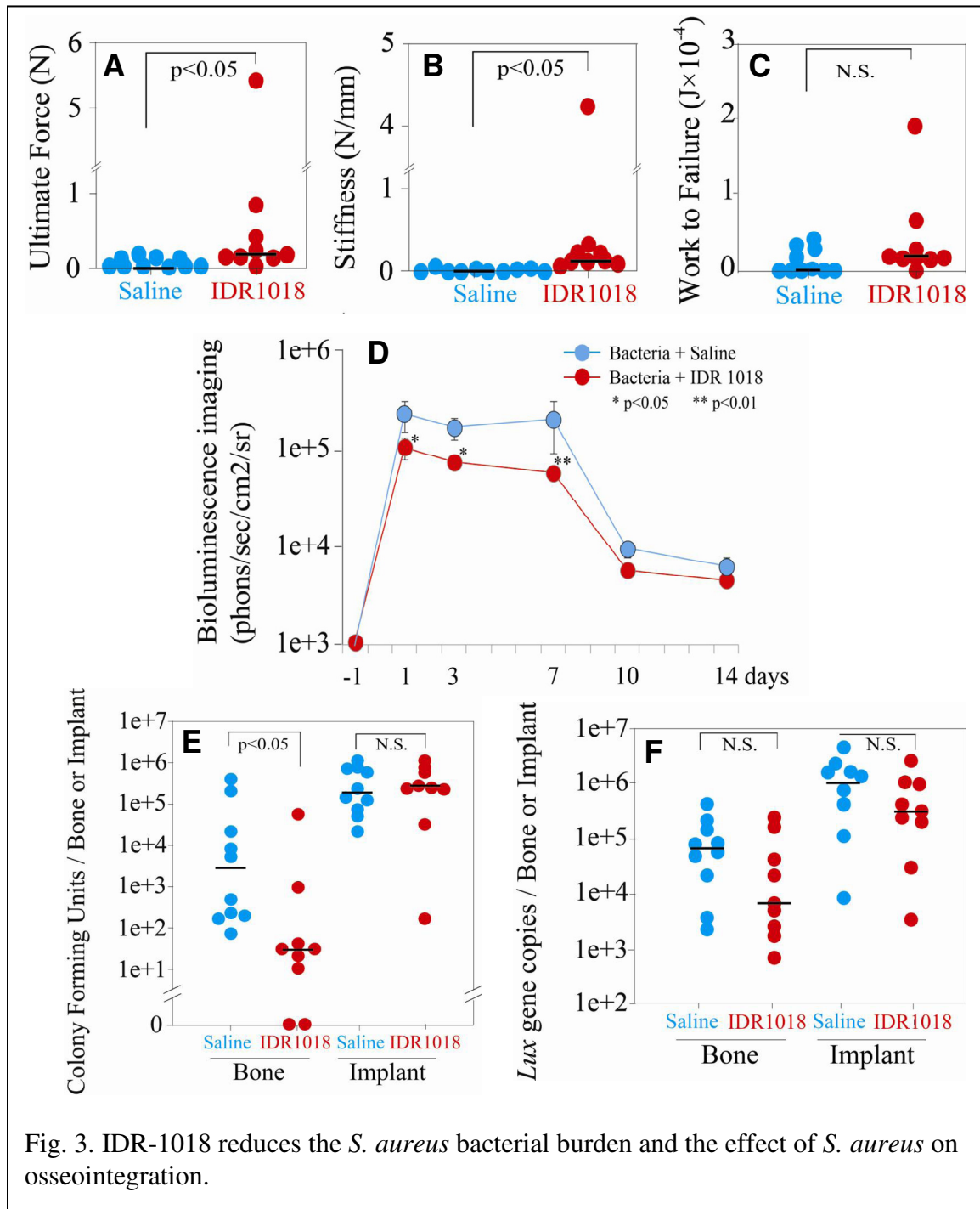
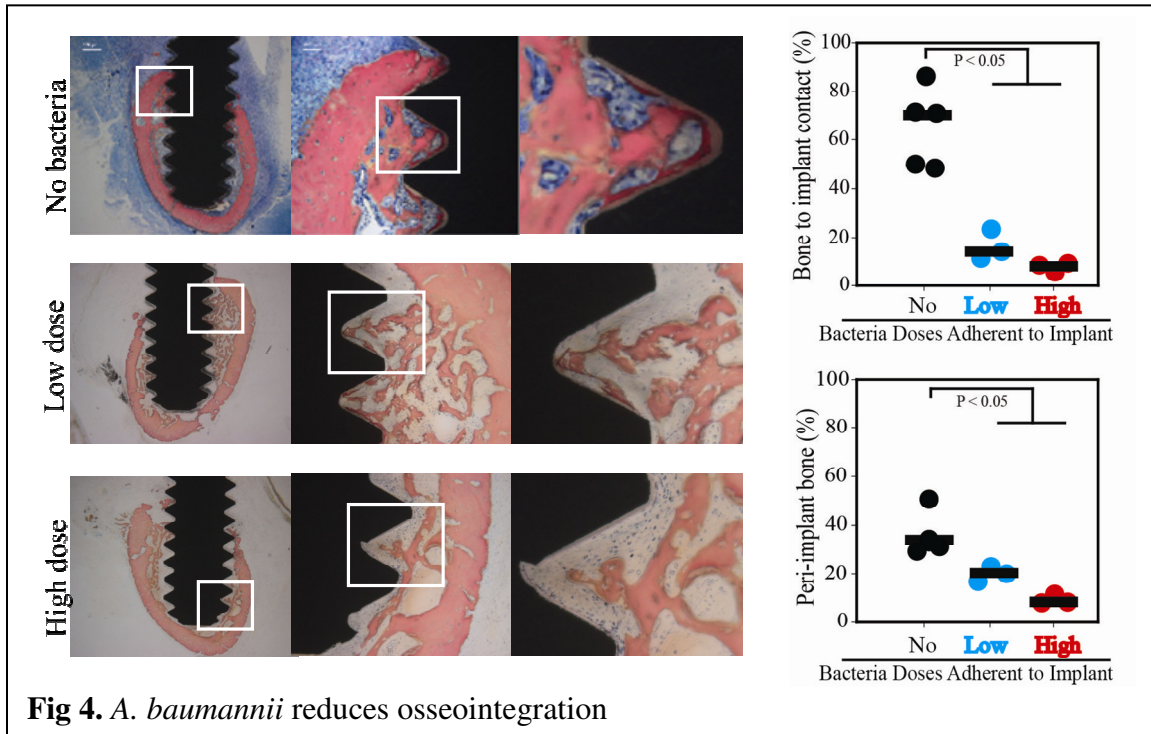


Fig. 3. IDR-1018 reduces the *S. aureus* bacterial burden and the effect of *S. aureus* on osseointegration.

The third primary area of progress is in developing a murine model of implant infection with *A. baumannii* Strain M2. Appropriate concentrations of *A. baumannii* were identified that reproducibly provide chronic implant infection without causing any signs of systemic sepsis. *A. baumannii* reduced osseointegration as assessed by histomorphometry (Fig. 4). Interestingly, preliminary observations suggest that the reduced bone formation appears to be limited to areas near to the implant, which would reconcile our results with previous murine studies of *A. baumannii* that did not detect bone loss [2,3]. Our results therefore demonstrate the importance of using a murine model of osseointegration.



KEY RESEARCH ACCOMPLISHMENTS:

1. The soluble host defense peptide increases production of MCP-1 but decreases the ability of LPS to induce TNF α or MCP-1 (Figs. 1-2).
2. The soluble host defense peptide reduces effects of *S. aureus* on orthopaedic implant osseointegration (Fig. 3).
3. Development of a quantitative and reproducible murine model of *A. baumannii* orthopaedic implant infection (Fig. 4).

REPORTABLE OUTCOMES:

Key Research Accomplishment #1 has not been reported.

Key Research Accomplishment #2 was reported in an oral presentation at the Military Health System Research Symposium, August 2013, Ft Lauderdale, Florida and in a poster at the Annual Meeting of the American Society for Bone and Mineral Research, October 2013, Baltimore MD (see Appendix)

Key Research Accomplishment #3 was reported in an oral presentation at the Military Health System Research Symposium, August 2013, Ft Lauderdale, Florida.

CONCLUSION:

The host defense peptides have the potential to substantially reduce infections of fractures sustained on the battlefield and in civilian settings. If the synthetic peptide reduces infections in the studies proposed in this application, more extensive pre-clinical testing would precisely determine its potential benefits and risks and determine whether the peptide is a high priority for human trials.

REFERENCES:

- [1] Wieczorek et al, Chem Biol. 2010 17:970-80
- [2] Collinet-Adler et al, Clin Orthop Relat Res. 2011 469:274-82
- [3] Crane et al, J Orthop Res. 2009 27:1008-15

APPENDIX: Poster presented at the Annual Meeting of the American Society for Bone and Mineral Research, October 2013, Baltimore MD

IDR-1018: A Synthetic Host Defense Peptide that Decreases Infection of Orthopaedic Implants

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Introduction

Orthopaedic implant infection is becoming increasingly difficult to treat due to the prevalence of multiple-drug-resistant bacteria. Host defense peptides are less likely than antibiotics to induce bacterial resistance because they decrease infection primarily by recruiting and activating macrophages rather than by directly killing the bacteria [1]. IDR-1018 is a novel small synthetic host defense peptide that decreases soft tissue infections [2 and 3].

The purpose of the current study was to determine whether IDR-1018 decreases implant infection and would therefore prevent impaired osseointegration due to infection.

Materials and Methods

Bacteriostatic and bactericidal effects on *Staphylococcus aureus*

The minimal inhibitory and minimal bactericidal concentration (MIC and MBC) of IDR-1018 on *S. aureus* were determined in both Mueller Hinton Broth and physiological saline.

Effects on implant infection:

- Bioluminescent *S. aureus* (Xen36 strain, Caliper Life Sciences) were adhered to titanium alloy implants (1 mm diameter, 3.2 mm length) before insertion into pilot holes in the mid-diaphysis of the femur of 6-8 week old mice [4 and 5].
- IDR-1018 (200ug/injection) or saline was administrated i.p. daily beginning 4 hours before implant insertion and 24 and 48 hours after implant insertion.
- Mice were euthanized at 15 days post operation.
- Bacterial burden was quantified by non-invasive bioluminescence imaging (BLI: Xenogen IVIS 200 system) as well as by counting colony forming units (CFUs) and *Lux* gene on the implants and in the bones.
- Osseointegration was measured by biomechanical pull-out testing as previously described for this murine implant model [4 and 5].

Results

Fig 1. IDR-1018 Reduces Bacterial Burden

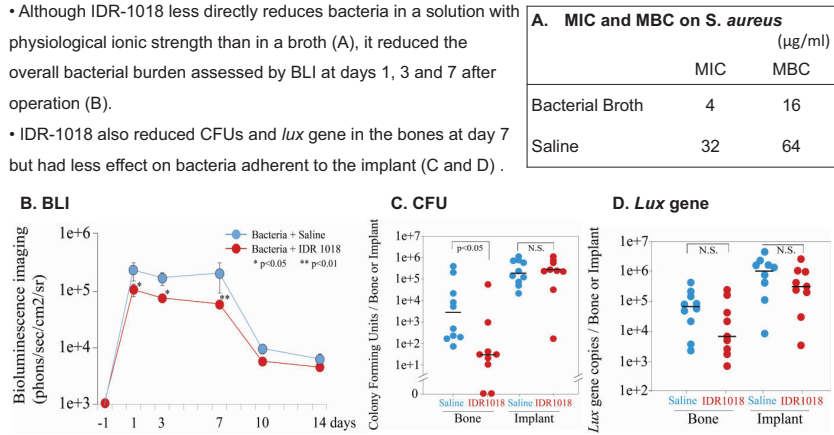
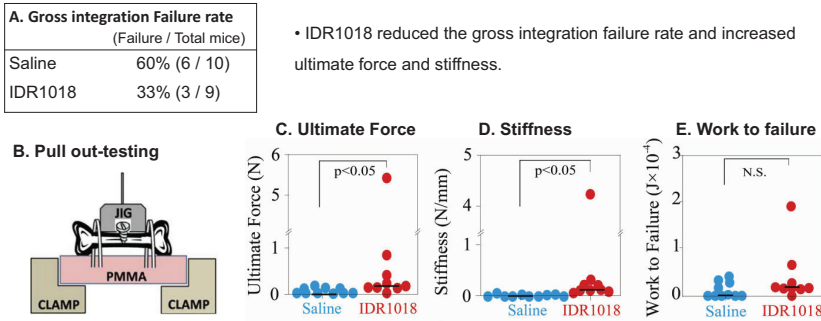


Fig 2. IDR-1018 Increases Osseointegration



Discussion

IDR-1018

- reduced implant infection and therefore prevented impaired osseointegration (Fig 1 and 2).
- has less effect on the bacteria adherent to the implant because biofilm-formation on the implant likely protect the bacteria from the antibacterial effect of IDR1018.
- potentially acts by recruiting and/or activating inflammatory cells [3] rather than by directly killing the bacteria in physiological ionic strength (Fig1.A) and therefore is unlikely to induce bacterial resistance.

Future study

IDR-1018 may be more effective in combination with antibiotics and/or implant removal. Evaluation of these possibilities is especially important because IDR-1018 is likely to be clinically used in combination with both antibiotics and implant removal.

Acknowledgement

1. Dept of Defense, Peer Reviewed Orthopaedic Research Program Idea Development Award
2. Mochida Memorial Foundation for Medical and Pharmaceutical Research

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