Additive Effects of Exogenous IL-12 Supplementation and Antibiotic Treatment in Infection Prophylaxis

Brandon M. Boyce,[1] Brock A. Lindsey,[1] Nina B. Clovis,[1] E. Suzanne Smith,[1] Gerald R. Hobbs,[2] David F. Hubbard,[1] Sanford E. Emery,[1] John B. Barnett,[3] Bingyun Li[1,4]

[1]Biomaterials, Bioengineering & Nanotechnology Laboratory, Department of Orthopaedics, School of Medicine, West Virginia University, Morgantown, West Virginia 26506, [2]Department of Statistics, West Virginia University, Morgantown, West Virginia 26506, [3]Department of Microbiology, Immunology, and Cell Biology, West Virginia University, Morgantown, West Virginia 26506, [4]WVNano Initiative, Morgantown, West Virginia 26506

Received 12 May 2011; accepted 13 July 2011
Published online 3 August 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.21520

ABSTRACT: The increasing clinical incidence and host risk of open fracture-associated infections, as well as the reduced effectiveness of conventional antibiotics to treat such infections, have driven the development of new therapies for the prophylaxis of open fracture-associated infections. We investigated percutaneous supplementation of a natural cytokine (i.e., interleukin 12p70 or IL-12) at an open fracture site to reduce open fracture-associated infections. We also determined the efficacy of the combination therapy of IL-12 and conventional antibiotic therapy in the prophylaxis of open fracture-associated infections. An open femur fracture infection model was produced by direct inoculation of a clinical isolate of *Staphylococcus aureus* after creating a femur fracture using rats. The animals were assigned to one of four groups: no drug administration, percutaneous supplementation of IL-12, intraperitoneal administration of the antibiotic ampicillin, or percutaneous IL-12 in combination with intraperitoneal ampicillin. Animals were euthanized at postoperative days 6, 10, 14, and 21. Percutaneous IL-12 led to a reduction in infection at postoperative days 6 and 10. For the first time, exogenous IL-12 was found to have additive effects in the prevention of infection when combined with conventional treatment (i.e., antibiotic therapy). Combination therapy of ampicillin and IL-12 substantially reduced the infection rate at postoperative day 6 and also decreased the time needed for complete inhibition of infection. Therefore, exogenous IL-12, providing a mechanism of protection independent of antibiotic resistance, complements the routine use of antibiotics. J Orthop Res 30:196–202, 2012

Keywords: infection prophylaxis; cell-mediated immune response; cytokine; antibiotic; percutaneous

Open fracture-associated infection is a major clinical complication affecting millions of people annually. It is estimated that approximately two million fracture fixation devices are implanted annually in the U.S.[1] and the incidence of infection after internal fixation may exceed 30%.[2,3] Meanwhile the incidence of open fractures is increasing due to increased survivability of high energy trauma in civilian settings as well as the military conflicts in Iraq, Afghanistan, and other countries. Antibiotic therapy is still primarily used for preventing open fracture-associated infections; however, overuse of antibiotics may lead to antibiotic resistance, which has become a worldwide issue.[4] Strains of *Staphylococcus aureus* (*S. aureus*) that resist or have reduced susceptibility to antibiotics such as methicillin and vancomycin have emerged.[5–7] According to the U.S. Centers for Disease Control and Prevention, more than 70% of the bacteria that cause hospital-acquired infections are resistant to at least one of the drugs most commonly used to treat them. Also, it was reported that more than 90,000 Americans contract potentially deadly infections each year from antibiotic-resistant *S. aureus* and related deaths may exceed those caused by acquired immune deficiency syndrome (AIDS).[8] Screening hospitalized patients by means of active surveillance cultures, aimed at controlling antibiotic-resistant pathogens, has met challenges such as limited resources and the potentially negative aspects of patient isolation.[9] A better prevention measure would be to curb the overuse of antibiotics.

Patients with traumatic open fractures or other serious injuries are vulnerable to infections, and it is generally agreed that the decreased resistance to infection is associated with abnormalities of both natural and adaptive immunity. Among those abnormalities is the loss of function of the T helper 1 (Th1) lymphocyte phenotype.[10–21] Because Th1 cells are principal regulators of cell-mediated immune response and the production of complement-fixing antibodies,[22] depressed function of this lymphocyte subset is believed to be related to reduced resistance to infection in injured patients.[12,16,19,21] Indeed, our previous studies that were designed to restore Th1 function, decreased due to major injuries, immediately after open fracture have shown increased resistance to infection.[23–25]

Interleukin 12p70 (IL-12), a natural cytokine, plays a central role in cell-mediated immune response and bridges innate and adaptive immunities;[26–28] therefore, it may contribute to infection prevention. In our previous studies, we incorporated IL-12 into polypeptide nanocoatings on implants, achieved sustained release of IL-12, and significantly reduced open fracture-associated infection.[23,24] In this study, we

Additional Supporting Information may be found in the online version of this article.

Correspondence to: Bingyun Li (T: 1-304-293-1075; F: 1-304-293-7070; E-mail: bli@hsc.wvu.edu).

determined whether IL-12, injected percutaneously, would enhance the host's resistance to infection in our rat open femur fracture model. We were especially interested in examining, for the first time, whether IL-12 and antibiotic treatments would have additive effects in reducing open fracture-associated infection.

MATERIALS AND METHODS

Open Fracture Model and Animal Groups

Approval for in vivo studies was obtained from our Institutional Animal Care and Use Committee and all procedures were performed under sterile conditions. *S. aureus*, which was isolated from a patient with chronic osteomyelitis, was used to create an open fracture infection model. A susceptibility test showed that the *S. aureus* was susceptible to gentamicin, chloramphenicol, vancomycin, cefazolin, nitrofurantoin, linezolid, rifampin, quinupristin, trimeth, doxycycline, minocycline, and tetracycline, and it was resistant to penicillin. Open fracture-associated infection in Sprague–Dawley rats was induced according to a previously reported protocol[23,24] and the surgical procedures are shown in Figure 1. In brief, each rat was anesthetized using Nembutal 50 mg/ml (Ovation Pharmaceuticals, Deerfield, IL) at a dose of 0.1 ml/100 g of body weight for an initial dose and then a booster of 0.2 ml 1 h following the initial dose. One of the rat's femurs was fractured using a custom-designed setup (Fig. 1a), the operative area was prepped, and an incision was made to expose the fracture (Fig. 1b). The fracture was inoculated with 100 μl 10^2 CFU/0.1 ml *S. aureus* (Fig. 1c) and left open for 1 h, mimicking the "golden hour" (broadly defined as the first 60 min following trauma or the onset of acute illness) of trauma patients. The fracture was then fixed with an intramedullary stainless steel Kirschner wire (K-wire, Fig. 1d) (Smith & Nephew, Memphis, TN). IL-12 was injected percutaneously at the fracture site (Fig. 1e). Buprenorphine HCl 0.3 mg/ml (Reckitt Benckiser Pharmaceuticals, Inc., Richmond, VA) was used for postoperative pain control at a dose of 0.03 mg/kg. The first dose was given preoperatively and it was given every 12 h through the first postoperative day. A total of four groups were studied (six rats for each time period per group): (1) control (designated as Control)—animals were fractured, infected, left open for 1 h, and then fixed; (2) percutaneous IL-12 therapy (designated as PIL)—animals were fractured, infected, left open for 1 h, and fixed, followed by percutaneous injection of IL-12 once daily for 10 doses (except the 6-day group; 50 ng/dose); (3) antibiotic therapy (designated as Antibiotic)—according to the standard of care for open fracture treatment, ampicillin trihydrate was given intraperitoneally immediately before fracture fixation and once daily for 2 days postoperatively; and (4) combination therapy of antibiotic and percutaneous IL-12 (designated as AntiPIL)—intraperitoneal injection of ampicillin trihydrate and percutaneous injection of IL-12 were both used. At postoperative days 6, 10, 14, or 21, the rats were anesthetized using Nembutal 50 mg/ml (Ovation Pharmaceuticals) at a dose of 0.1 ml/100 g of body weight and then euthanized by administering 1 cc of Euthasol (Virbac AH, Inc., Fort Worth, TX) via intracardiac puncture.

For each animal group, if no infection was seen at the 6-day timepoint, then the 10- and 14-day timepoints were omitted. As a result, the Control, Antibiotic, and PIL groups were euthanized at postoperative days 6, 10, 14, and 21, and the AntiPIL group at postoperative days 6 and 21. Weight was recorded before surgery as well as before euthanasia. Note that two animals in the Antibiotic 10-day group died at postoperative day 0 due to anesthesia and one animal in the Antibiotic 6-day group was excluded after a comminuted femur fracture at postoperative day 0.

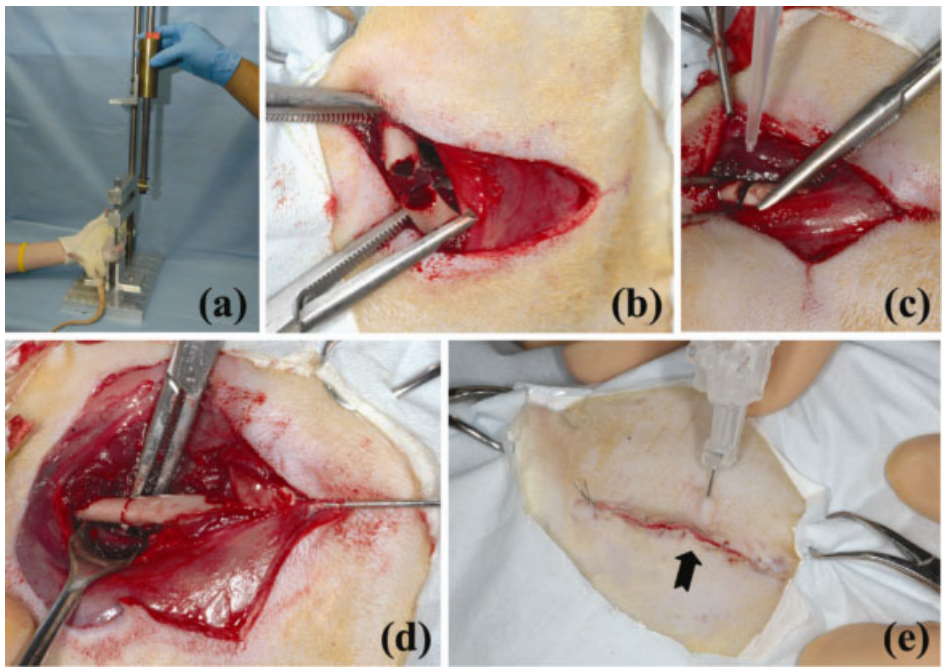

Figure 1. Procedures in creating an open femur fracture and subsequent percutaneous IL-12 injection. (a) Creation of a femur fracture using a custom-designed setup; (b) exposure of the fracture; (c) bacterial inoculation; (d) intramedullary fixation of the fracture using a K-wire; and (e) percutaneous injection of IL-12. The arrow shows the fracture site.

Microbiological Evaluations

As previously reported, infection was determined based on bacterial culturing of the surgical femur and blood samples.[23,24,29] Briefly, the surgical femur was sterilely removed following euthanasia and approximately 500 mg of femur was homogenized in brain heart infusion broth (Becton Dickinson, Cockeysville, MD). The ratio of bone to broth was kept at 100 mg to 1 ml; 100 μl of the homogenized bone broth was plated onto blood agar plates and placed in a 37°C incubator for 48 h. Infection was defined as the presence of >2 to 5 bacterial colonies per plate. Blood samples, collected before euthanasia, were also plated.

Blood Analyses

Immediately before euthanasia, blood was collected from each animal and used for complete blood count and flow cytometry studies (BD FACSCalibur Flow Cytometer, BD Biosciences, Franklin Lakes, NJ). Complete blood count samples were analyzed by Antech Diagnostics, Inc. (Southaven, MS) for blood cell analysis. Flow cytometry was used to analyze serum samples by staining the cells with purified anti-rat mononuclear phagocyte antibody (1C7) followed by Cy-5 labeled goat anti-mouse IgG and FITC labeled anti-rat MHC class II (OX-6). The relative intensity of MHC II on 1C7 positive cells represents the degree of macrophage activation.

Statistical Analyses

Data were expressed as the mean ± standard deviation (SD). Differences were analyzed using ANOVA. Post hoc comparisons among means were done with Tukey's HSD procedure. $p < 0.05$ was considered statistically significant. JMP (V9) software was used (SAS Institute, Inc., Cary, NC).

RESULTS

In this study, we investigated the effectiveness of percutaneous IL-12 and the combination of IL-12 with conventional antibiotic treatment in preventing open fracture-associated infection.

Effects of Antibiotic, Percutaneous IL-12, and Their Combination on Infection

Quantitative culturing of bone tissue homogenates was carried out to determine infection rates (Table 1). We found that the "gold standard" antibiotic therapy decreased the infection rate from 100% in the Control group to 40%, 25%, and 0% at postoperative days 6, 10, and 21, respectively. Percutaneous IL-12 therapy reduced the infection rate from 100% of Control to 83% at postoperative days 6 and 10 but did not reduce the infection rate at postoperative days 14 and 21. The combination therapy of percutaneous IL-12 and antibiotic resulted in the best infection prevention outcome since the combination therapy completely prevented infection as early as postoperative day 6, compared to postoperative day 14 for the animals treated with antibiotic alone. In addition, no systemic infection was detected in any groups at the time periods studied as demonstrated by no bacterial growth in the blood samples.

Table 1. Infection Rates of Rats

	Infection Rate, %			
Rat Group	Day 6	Day 10	Day 14	Day 21
Control	100	100	100	100
PIL	83	83	100	100
Antibiotic	40[a]	25[b]	0	0
AntiPIL	0	—	—	0

—, Not done. Note that if no infection was found at any of the earlier postoperative days then the subsequent intermediate time periods were omitted.
[a]One animal excluded due to issues related to fracture process.
[b]Two died.

Weight Loss and Gross Observation

All the animal groups had less weight loss at postoperative day 21 compared to day 6 but there were no significant differences among all the groups at postoperative days 6 and 21, and significantly higher weight loss was observed in the PIL group compared to the Control and Antibiotic groups at postoperative days 10 and 14 (Fig. 2). Gross observation upon animal euthanasia showed better healing in the Antibiotic and AntiPIL groups than the Control and PIL groups at postoperative day 21. No obvious difference in gross observation of healing was observed between the Antibiotic group and the AntiPIL group.

Macrophage Activation and Systemic Responses

There was no significant difference in macrophage activation among all the animal groups at the same postoperative day (Fig. 3). Differences in the serum levels of white blood cells (WBCs), platelets, neutrophils, lymphocytes, and monocytes were observed (Fig. 4). A higher number of WBCs, platelets, and neutrophils were found at postoperative day 6 with lower levels at postoperative day 21 in the treatment groups (i.e., Antibiotic, PIL, and AntiPIL groups) compared to the Control group. During the time periods studied, the levels of WBCs, platelets, neutrophils, and lymphocytes in the PIL group increased from postoperative day 6 to postoperative day 10 and reached a maximum at postoperative day 14, after which, at postoperative day 21, their levels decreased. Among all groups, the

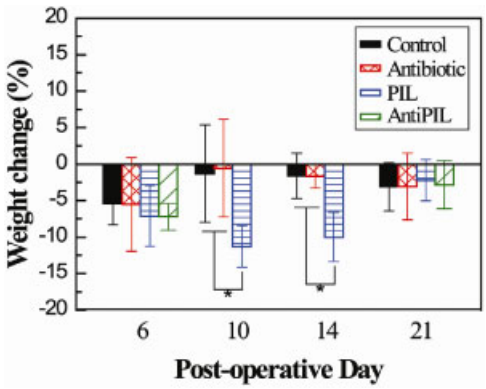

Figure 2. Weight loss of rat groups. Data are an average of 4–6 rats.

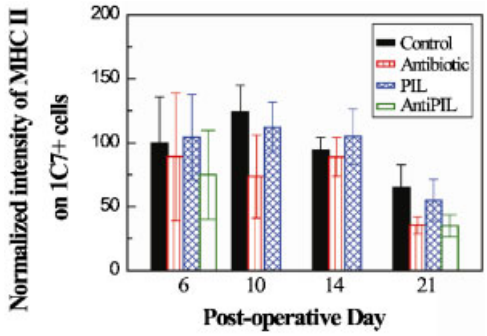

Figure 3. Normalized intensity of MHC II on 1C7+ cells. The intensity of the control group was set at 100.

PIL group had relatively higher levels of WBCs, neutrophils, lymphocytes, and monocytes at postoperative days 6, 10, and 14. The PIL group had significantly higher WBCs, neutrophils, and monocytes compared to the Control and Antibiotic groups at postoperative day 14 (Fig. 4a,c,e). No major significant differences in WBCs, platelets, lymphocytes, and monocytes were noted within the Control, Antibiotic, PIL, and AntiPIL groups at postoperative day 21 (Fig. 4a,b,d,e). Significantly higher neutrophils were observed in the Control group compared to the Antibiotic and AntiPIL groups at postoperative day 21 (Fig. 4c). In addition, no significant difference was found in hemoglobin, hematocrit, red blood cells, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration (see Supplementary Materials).

DISCUSSION

Trauma and major burns have been reported to result in diminished production of IL-12, reduced Th1 responses, and decreased resistance to infection.[10–21] IL-12 has multiple biologic effects on T-cell and natural killer (NK) cell functions. It stimulates NK cells to

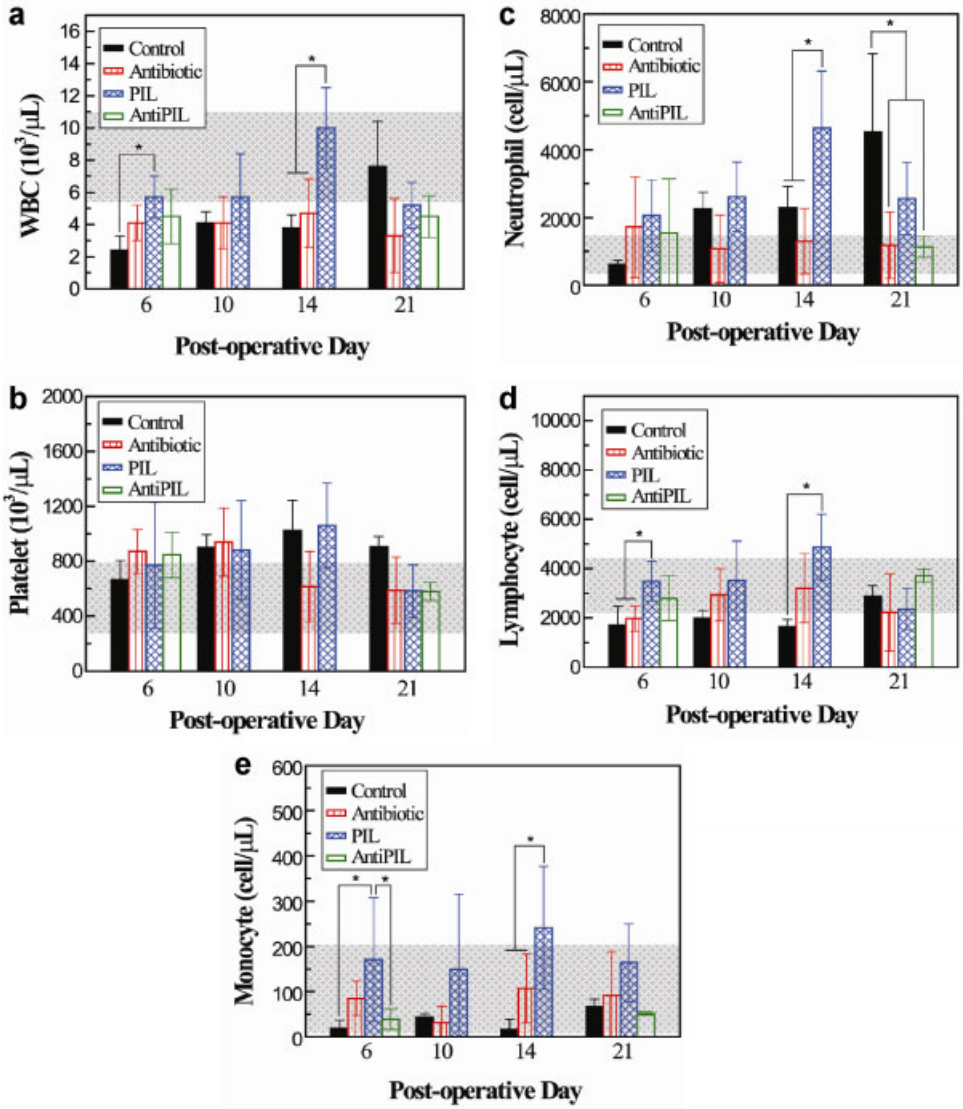

Figure 4. Systemic responses: (a) WBCs; (b) platelets; (c) neutrophils; (d) lymphocytes; and (e) monocytes. Shadowed areas indicate the reference levels.

release interferon γ (IFN-γ), induces type 1 helper T (Th1) cell and cytotoxic T lymphocyte proliferation, and promotes the progression of cell-mediated immunity. IL-12 appears to be requisite for generating optimal Th1 responses in many experimental settings.[30,31] Meanwhile, IL-12 may also contribute to the genesis of some forms of immunopathology, as reviewed in the literature.[32] We have presented here that the application of percutaneous IL-12 (a cytokine that induces expression of the Th1 phenotype) to the open femur fracture infection model reduced infection at postoperative days 6 and 10. Therefore, percutaneous IL-12 therapy applied to the open fracture has restored, to some degree, the capability of the host's natural resistance to infection.

Neutrophils provide the first line of defense against pathogen invasion. Increased levels of neutrophils were observed after intranasal administration of IL-12 for 48 h in a respiratory infection model,[33] where enhanced resistance against *Streptococcus pneumoniae* was due to enhanced IFN-γ-mediated neutrophil recruitment.[33] Similarly, the application of percutaneous IL-12 in this study led to higher levels of neutrophils at postoperative days 6, 10, and 14 compared to the Control and Antibiotic groups, which may have contributed to the enhancement of resistance at postoperative days 6 and 10. Macrophage activation, central to host defense against pathogenic organisms, is also compromised as a consequence of major injuries.[34,35] It is also possible that IL-12 treatment directly causes macrophages to become more phagocytic. Therapies such as IFN-γ treatment were reported to inhibit both intracellular and extracellular pathogens in vivo,[33,34,36,37] probably because IFN-γ can augment phagocyte Fc receptor expression as well as neutrophil antibody-dependent cell-mediated cytotoxicity, phagocytosis, and neutrophil recruitment.[33,34,37,38] IFN-γ coordinated neutrophil recruitment did not result in pathologic activation.[38] As a result, percutaneous IL-12 therapy reduced the infection rate at postoperative days 6 and 10 compared to the Control group. The combination therapy of IL-12 and antibiotic seemed to have additive effects on infection prevention and it substantially shortened the time needed for complete inhibition of infection (6 days) compared to antibiotic therapy alone (14 days) (Table 1).

The production of IL-12, together with several other Th1 cytokines, was significantly reduced soon after serious injury.[12,15,16,35] Exogenous application of IL-12 could restore the normal level of IL-12, which is important in restoring normal resistance to a bacterial challenge.[16] Use of IL-12 may not lead to prolonged inflammation or significant inflammatory tissue damage due to the facts that IL-12 production is significantly reduced soon after injury and cytokines like IL-12 have a short in vivo half-life. In this and our previous studies,[23,24] significant inflammatory tissue damage was not observed in the IL-12 treated groups compared to the other groups. However, significantly higher weight loss was observed in the PIL group compared to the Control and Antibiotic groups at postoperative days 10 and 14 although no significant differences were found on days 6 and 21. The reason for the higher weight loss on days 10 and 14 was not clear and may require further studies. Percutaneous IL-12 resulted in increased serum levels of WBCs, platelets, neutrophils, lymphocytes, and monocytes as postoperative time increased from days 6 to 14. However, at day 21, their levels returned to those at postoperative day 6 and were about the same as those treated with antibiotic (Fig. 4).

IL-12 therapy was found to be more effective, compared to IFN-γ and other therapies, in restoring normal resistance to a bacterial challenge in a mouse model of burn injury.[12] It is still uncertain as to the reasons IL-12 was more effective than IFN-γ; one possible reason was that IL-12 acts earlier in the cytokine cascade and is a potent stimulus of NK cell activity. Meanwhile, cytokines, including IL-12 and IFN-γ, have a short in vivo half-life of a few minutes or hours and IL-12 applied by percutaneous injection may undergo rapid degradation. This might explain why percutaneous IL-12 alone did not lead to complete inhibition of infection. An appropriate drug delivery system may improve IL-12 efficacy in prophylaxis of infection if the delivery system could substantially prolong its in vivo half-life. In the past we showed that IL-12 embedded in polypeptide nanocoatings on implants were effective in infection prophylaxis.[23] We had sustained release, up to 9 days, of IL-12 from polypeptide nanocoatings which led to a significant reduction of infection from 90% in Controls to 20% in IL-12 treated animals.[23] Importantly, in this study, we found that IL-12 and antibiotic treatment had additive effects in reducing infection associated with open fractures. Evaluation of the effect of IL-12 on bone healing is beyond the scope of this study and may be considered in the future.

In summary, we have shown that prophylactic treatment with percutaneous IL-12 reduced infection and exogenous IL-12 combined with conventional antibiotic treatments had additive effects in preventing open fracture-associated infections. The combination therapy has led to significantly earlier inhibition of infection. The use of locally administered IL-12 is complementary to current antibiotic therapy and the combination therapy of IL-12 and antibiotic is a potentially useful treatment modality against open fracture-associated infections.

ACKNOWLEDGMENTS

The authors acknowledge financial support from the Osteosynthesis and Trauma Care Foundation, NSF (#1003907), and WVU Research Corporation. The authors appreciate the use of the cytometer at the Flow Cytometry Core Facility at West Virginia University Health Sciences Center. We acknowledge John G. Thomas, PhD (Department of Microbiology, Immunology and Cell Biology) for the consultation on

microbiology. We thank Kathleen Brundage, PhD (Department of Microbiology, Immunology and Cell Biology) for assisting in flow cytometry studies.

REFERENCES

1. Darouiche RO. 2004. Treatment of infections associated with surgical implants. N Engl J Med 350:1422–1429.
2. Zalavras CG, Patzakis MJ. 2003. Open fractures: evaluation and management. J Am Acad Orthop Surg 11(3):212–219.
3. Schmidmaier G, Lucke M, Wildemann B, et al. 2006. Prophylaxis and treatment of implant-related infections by antibiotic-coated implants. Injury 37(S2):S105–S112.
4. Facts about antibiotic resistance (CDC): http://www.cdc.gov/getsmart/antibiotic-use/fast-facts.html. Accessed on November 20, 2010.
5. Heseltine P. 2000. Has resistance spread to the community? Clin Microbiol Infect 6(S2):11–16.
6. Fridkin SK, Hageman J, McDougal LK, et al. 2003. Epidemiological and microbiological characterization of infections caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin, United States, 1997–2001. Clin Infect Dis 36(4):429–439.
7. Jackson CR, Fedorka-Cray PJ, Davis JA, et al. 2009. Prevalence, species distribution and antimicrobial resistance of enterococci isolated from dogs and cats in the United States. J Appl Microbiol 107(4):1269–1278.
8. Klevens RM, Morrison MA, Nadle J, et al. 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 298:1763–1771.
9. Weber SG, Huang SS, Oriola S, et al. 2007. Legislative mandates for use of active surveillance cultures to screen for methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci: position statement from the Joint SHEA and APIC Task Force. Am J Infect Control 35:73–85, and Infect Control Hosp Epidemiol 28:249−260.
10. Wood JJ, Rodrick ML, O'Mahony JB, et al. 1984. Inadequate interleukin 2 production. A fundamental immunological deficiency in patients with major burns. Ann Surg 200(3):311–320.
11. Faist E, Mewes A, Baker CC, et al. 1987. Prostaglandin E2 (PGE2)-dependent suppression of interleukin alpha (IL-2) production in patients with major trauma. J Trauma 27(8) 837–848.
12. O'Sullivan ST, Lederer JA, Horgan AF, et al. 1995. Major injury leads to predominance of the T helper-2 lymphocyte phenotype and diminished interleukin-12 production associated with decreased resistance to infection. Ann Surg 222(4): 482–490; discussion 490−492.
13. Decker D, Schondorf M, Bidlingmaier F, et al. 1996. Surgical stress induces a shift in the type-1/type-2 T-helper cell balance, suggesting down-regulation of cell-mediated and up-regulation of antibody-mediated immunity commensurate to the trauma. Surgery 119(3):316–325.
14. Kelly JL, Lyons A, Soberg CC, et al. 1997. Anti-interleukin-10 antibody restores burn-induced defects in T-cell function. Surgery 122(2) 146–152.
15. Brune IB, Wilke W, Hensler T, et al. 1999. Downregulation of T helper type 1 immune response and altered pro-inflammatory and anti-inflammatory T cell cytokine balance following conventional but not laparoscopic surgery. Am J Surg 177(1):55–60.
16. Göebel A, Kavanagh E, Lyons A, et al. 2000. Injury induces deficient interleukin-12 production, but interleukin-12 therapy after injury restores resistance to infection. Ann Surg 231(2):253–261.
17. Boelens PG, Fonk JC, Houdijk AP, et al. 2004. Primary immune response to keyhole limpet haemocyanin following trauma in relation to low plasma glutamine. Clin Exp Immunol 136(2):356–364.
18. MacConmara MP, Maung AA, Fujimi S, et al. 2006. Increased CD4+ CD25+ T regulatory cell activity in trauma patients depresses protective Th1 immunity. Ann Surg 244(4):514–523.
19. Klehmet J, Harms H, Richter M, et al. 2008. Stroke-induced immunodepression and post-stroke infections: lessons from the preventive antibacterial therapy in stroke trial. Neuroscience 158(3):1184–1193.
20. Hsieh CH, Hsu JT, Hsieh YC, et al. 2009. Suppression of activation and costimulatory signaling in splenic CD4+ T cells after trauma-hemorrhage reduces T-cell function: a mechanism of post-traumatic immune suppression. Am J Pathol 175(4):1504–1514.
21. Held KS, Steward O, Blanc C, et al. 2010. Impaired immune responses following spinal cord injury lead to reduced ability to control viral infection. Exp Neurol 226(1):242–253.
22. Mosmann TR, Sad S. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 17(3):138–146.
23. Li B, Jiang B, Boyce BM, et al. 2009. Multilayer polypeptide nanoscale coatings incorporating IL-12 for the prevention of biomedical device-associated infections. Biomaterials 30: 2552–2558.
24. Li B, Jiang B, Dietz MJ, et al. 2010. Evaluation of local MCP-1 and IL-12 nanocoatings for infection prevention in open fractures. J Orthop Res 28:48–54.
25. Li B, McKeague AL. 2010. Emerging ideas: interleukin-12 nanocoatings prevent open fracture-associated infections. Clin Orthop Relat Res. DOI: 10.1007/s11999-010-1690-0.
26. Trinchieri G, Wysocka M, D'Andrea A, et al. 1992. Natural killer cell stimulatory factor (NKSF) or interleukin-12 is a key regulator of immune response and inflammation. Prog Growth Factor Res 4(4):355–368.
27. Trinchieri G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 13:251–276.
28. Méndez-Samperio P. 2010. Role of interleukin-12 family cytokines in the cellular response to mycobacterial disease. Int J Infect Dis 14(5):e366–e371.
29. Lucke M, Wildemann B, Sadoni S, et al. 2005. Systemic versus local application of gentamicin in prophylaxis of implant-related osteomyelitis in a rat model. Bone 36(5): 770–778.
30. Magram J, Connaughton SE, Warrier RR, et al. 1996. IL-12-deficient mice are defective in IFN gamma production and type 1 cytokine responses. Immunity 4(5):471–481.
31. Cooper AM, Magram J, Ferrante J, et al. 1997. Interleukin 12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with mycobacterium tuberculosis. J Exp Med 186(1):39–45.
32. Gately MK, Renzetti LM, Magram J, et al. 1998. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. Annu Rev Immunol 16:495–521.
33. Sun K, Salmon SL, Lotz SA, et al. 2007. Interleukin-12 promotes gamma interferon-dependent neutrophil recruitment in the lung and improves protection against respiratory *Streptococcus pneumoniae* infection. Infect Immun 75(3): 1196–1202.
34. Dries DJ, Jurkovich GJ, Maier RV, et al. 1994. Effect of interferon gamma on infection-related death in patients with severe injuries. A randomized, double-blind, placebo-controlled trial. Arch Surg 129(10):1031–1041; discussion 1042.

35. Flohé SB, Agrawal H, Flohé S, et al. 2008. Diversity of interferon gamma and granulocyte-macrophage colony-stimulating factor in restoring immune dysfunction of dendritic cells and macrophages during polymicrobial sepsis. Mol Med 14(5–6):247–256.
36. Maheshwari RK, Czarniecki CW, Dutta GP, et al. 1986. Recombinant human gamma interferon inhibits simian malaria. Infect Immun 53(3):628–630.
37. Petroni KC, Shen L, Guyre PM. 1988. Modulation of human polymorphonuclear leukocyte IgG Fc receptors and Fc receptor-mediated functions by IFN-gamma and glucocorticoids. J Immunol 140(10):3467–3472.
38. Bonville CA, Percopo CM, Dyer KD, et al. 2009. Interferon-gamma coordinates CCL3-mediated neutrophil recruitment in vivo. BMC Immunol 10:14.