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EMERGING IDEAS

Emerging Ideas

Interleukin-12 Nanocoatings Prevent Open Fracture-associated Infections

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Abstract
Background Infection is a major clinical complication of orthopaedic implants and prosthetic devices, and patients with traumatic open fractures have a high risk of infection that may exceed 30%. Surgical trauma, burns, and major injuries such as traumatic open fractures induce immunosuppression, decrease resistance to infection, and decrease production of T helper type 1 (Th1) cytokines.
Questions/hypotheses Exogenous interleukin-12 p70 (IL-12p70 or IL-12), a natural cytokine that plays a central role in Th1 response and bridges innate and adaptive immunities, will reduce open fracture-associated infection.
Method of Study We propose using exogenous IL-12 nanocoating to restore or enhance the body's natural defense system to combat pathogens. Rats will have a femur fractured, inoculated with Staphylococcus aureus or injected with phosphate buffered saline, left open for 1 hour, and then fixed with an intramedullary Kirschner wire with or without IL-12 nanocoating. Animals will be euthanized at postoperative Day 21; samples of blood, soft tissue, bone, and draining lymph nodes will be collected. Infection, bone healing, and local and systemic responses will be determined.
Significance IL-12 nanocoating is a promising prophylactic means to modulate the host immune response to help prevent open fracture-associated infections and to avoid the problem of antibiotic resistance.

Hypothesis

Exogenous IL-12, a natural cytokine that plays a central role in Th1 response and bridges innate and adaptive immunities, will reduce open fracture-associated infection.

Background

Open fracture-associated infections are major problems which may lead to prolonged hospitalization, sepsis, poor functional outcome, and even death [8, 23, 25]. Every year,

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more than one million Americans are hospitalized for bone fractures [5], and such injuries are increasingly common because of increased survivability of high-energy trauma in civilian settings and in military conflicts. Patients with traumatic open fractures have a high risk of infection, and this is further complicated by the increasing emergence of virulent and multidrug-resistant bacterial strains.

Injury plays an important role in host immune response. Accumulating clinical observations and experimental studies indicate that surgical trauma, burns, or injuries cause transiently decreased Th1-type responses, which are thought to be responsible for the decreased resistance to infections [1, 3, 15, 19]. Injury-suppressed immune responses may include suppressed IL-12 production by macrophages and dendritic cells [11, 14, 22] and reduced interferon-γ (IFN-γ) production [4, 9, 13, 18, 22].

IL-12 (also termed IL-12 p70) plays a central role in promoting Th1 responses [6, 10, 12, 26, 27], and displays multiple biologic effects on T-cell and natural killer (NK) cell functions (Fig. 1A). Any deficiency in IL-12 production may result in impairment of various immunologic functions. Local administration of exogenous IL-12 may create an environment rich in IL-12 locally and may result in enhanced Th1 reactivity and early activation of macrophages to prevent infection (Fig. 1B). An increased understanding of how local IL-12 treatments affect local and systemic immune responses may reveal new insights into ways in which normal immune function could be restored to resist common pathogens after injury.

Proposed Program

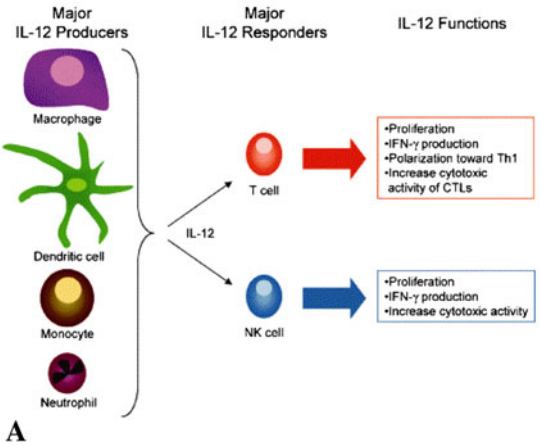
In our preliminary studies, we developed an open femur fracture infection model using Sprague-Dawley rats. We showed IL-12 nanocoatings substantially decreased the infection rates on postoperative Day 21 (Table 1) [16, 17]. To further determine the feasibility of using IL-12 for infection prevention, we propose to determine the therapeutic and side effects (if any) of IL-12 nanocoating treatments and explore its mechanism of action in preventing open fracture-associated infections.

We will determine the cellular and inflammatory mediator responses and show that IL-12 nanocoating induces Th1 activation and results in a decrease in infection. We will determine the effects of IL-12 nanocoating on local and systemic responses of immune cells (eg, Th1 cells, B cells, NK cells, and macrophages) and Th1 and Th2 cytokine production and correlate the change in immune cells and cytokine production with infection outcome. The working hypothesis is that IL-12 nanocoating

Table 1. Infection percentage in rats with different treatments at postoperative Day 21

IL-12 nanocoating (10.6 ng)	Control	Systemic IL-12		
		(10 ng)	(200 ng)	(1000 ng)
20%	90%	100%	100%	100%

IL-12 = interleukin-12.



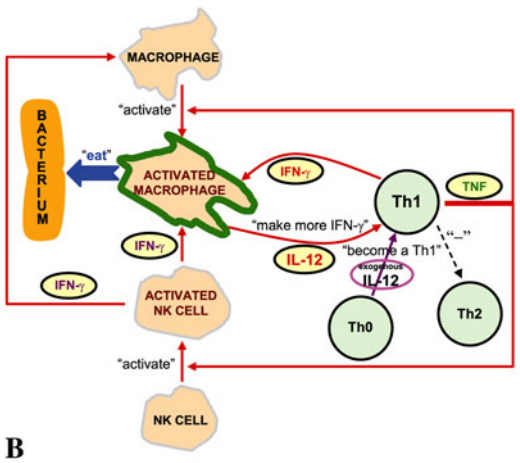


Fig. 1A–B (**A**) Cellular sources and responders to IL-12 are shown. Antigen-presenting cells and phagocytic cells, including monocytes and macrophages, dendritic cells, and neutrophils, are the primary producers of IL-12. The major actions of IL-12 are on T and NK cells. IL-12 induces proliferation, interferon-γ (IFN-γ) production, and increased cytotoxic activity of these cells, and importantly, IL-12 induces the polarization of $CD4^+$ T cells to the Th1 phenotype that mediates immunity against intracellular pathogens. IL-12, especially in combination with IL-18, also acts on macrophages and dendritic cells to induce IFN-γ production, even in antigen-presenting cells. (Reprinted from Watford WT, Moriguchi M, Morinobu A, O'Shea JJ. The biology of IL-12: coordinating innate and adaptive immune responses. *Cytokine Growth Factor Rev*. 2003;14:361-368. Copyright (2003) with permission from Elsevier.) (**B**) Local IL-12 therapy stimulates Th cells to secrete Th1 cytokines. Through positive feedback, Th1 immunity can be promoted to battle bacteria such as Staphylococcus aureus. (Reprinted from Li B, Jiang B, Boyce BM, Lindsey BA. Multilayer polypeptide nanoscale coatings incorporating IL-12 for the prevention of biomedical device-associated infections. *Biomaterials*. 2009;30: 2552−2558. Copyright (2009) with permission from Elsevier.)

treatment will restore the IL-12 level that is decreased as a result of trauma and activate the Th1 pathway that is important in host defense thereby preventing infection.

We also will determine the therapeutic and potential side effects of IL-12 nanocoating on uninfected and S. aureus-infected animals with open fractures. The effect of IL-12 nanocoating on uninfected and S. aureus-infected animals will be examined in an open femur facture rat model. We hypothesize that IL-12 nanocoating treatment prevents infection and does not lead to substantial inflammatory tissue damage, and IL-12 nanocoating increases the production of Th1 mediators important in host defense and at the same time maintains the production of Th2 cytokines such as IL-10, an important antiinflammatory cytokine.

We expect to determine the effects of IL-12 nanocoating on infected and uninfected animals, and we expect a better understanding of tuning Th1 response in preventing open fracture-associated infections.

Limitations

In our preliminary studies, we observed no major toxicity in rats treated with IL-12. Several studies also have showed treatments with 1000 ng/kg IL-12 in humans [20] and mice [21] were well tolerated. Local delivery of the IL-12 gene may be used as an alternative to the IL-12 protein; local IL-12 gene delivery showed very limited or no toxicity [7]. Autogenous platelet-rich plasma (PRP) also may be used as an alternative to IL-12 therapy; PRP may avoid inflammatory tissue damage (not observed so far). According to one report, autogenous PRP inhibited the growth of S. aureus and topical application of PRP in the treatment of chronic femoral osteomyelitis appeared to promote healing and prevent infection [2]. The proposed exogenous IL-12 is preferred because the sources of autogenous PRP are limited and PRP treatments usually require multiple blood draws from the same patient. Finally, one potential concern of IL-12 therapy is its cost. Local administration, compared with systemic applications, requires substantially less IL-12 and its cost is acceptable.

Next Steps

Our preliminary studies suggest IL-12 nanocoatings may prevent infection associated with implants [16, 17]. IL-12 nanocoatings provide the drug at the implant/tissue interface thereby maximizing the exposure of the drug to potentially contaminated tissues. In the future, we will determine whether combination therapy of IL-12 and antibiotic will be a potentially useful approach against open fracture-associated infections and whether the combination of IL-12 and antibiotic will have synergetic effects in reducing infection. Moreover, we may incorporate IL-12 and growth factors (eg, insulin-like growth factor-1 [IGF-1] or bone morphogenetic protein-2 [BMP-2]) in the nanocoating system to further enhance bone healing while preventing infection. Incorporation of growth factors (eg, IGF-1or BMP-2) has the potential to further enhance bone healing [24].

Moreover, we may consider applying the developed local IL-12 therapies to a large animal model such as a goat open tibia fracture model. One major barrier in the translation of therapies from rodents to humans is the relatively advanced maturity of the human immune system. This difference is believed to be a major reason why many promising therapies fail to translate to more complex animals. Determining the effect of IL-12 treatments on infection reduction and the importance of related immune response in a goat model would be necessary to better reflect the human condition. If promising, clinical trials and FDA approval will be pursued.

Vision of the Future

On completing these studies, there is the hope that an effective prophylactic measure would be available, thus reducing the need for costly postoperative treatment of open fracture-associated infections. The use of locally administered IL-12 could be complementary to current antibiotic therapy and potentially could be a useful extension of therapy against open fracture-associated infections.

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