IL-33 promotes Staphylococcus aureus-infected wound healing in mice

Hui Yin, Xiangyong Li, Shilian Hu, Tao Liu, Baohong Yuan, Qian Ni, Fang Lan, Xiaochun Luo, Hongbiao Gu, Fang Zheng

A R T I C L E   I N F O

Article history:
Received 10 April 2013
Received in revised form 28 June 2013
Accepted 12 July 2013
Available online xxxx

Keywords:
IL-33
Bacteria
Infection
Neutrophil
Wound healing

A B S T R A C T

Interleukin (IL)-33, a newly identified member of IL-1 family of cytokines, plays a crucial role in polarizing Th2-associated immune responses. Recently growing evidence indicates that IL-33 also represents an important mediator of mucosal healing and epithelial restoration. In this study, we investigated the effect of IL-33 on antimicrobial infection and wound healing using a Staphylococcus aureus-infected incision model in mice. Our findings showed that the expression of IL-33 mRNA and protein was promptly increased in cutaneous wound tissues when challenged by methicillin-resistant S. aureus (MRSA). At the same time, exogenous IL-33 administration profoundly inhibited MRSA colonization and accelerated cutaneous wound repair. IL-33 upregulation promoted the proliferation of neutrophils and CXCR2 expression, which is related to augmented neutrophil trafficking into infectious sites for bactericidal effect. In addition, the beneficial effect of IL-33 is also implicated in enhancement of collagen deposition and the expression of extracellular matrix (ECM)-associated genes such as fibronectin and collagen Ila, which implies a potential effect of IL-33 on matrix synthesis and reepithelialization during the wound repair process. All together, these findings reveal that IL-33 plays an essential effect in maintenance of cutaneous homeostasis and improvement of infectious wound healing.

© 2013 Published by Elsevier B.V.

1. Introduction

Staphylococcus aureus is responsible for the majority of skin and soft tissue infections, including impetigo, folliculitis and cellulitis [1,2]. In addition, this bacterium can also cause invasive and life-threatening infections, such as abscesses, osteomyelitis, pneumonia, endocarditis, meningitis and sepsis [3–5]. These infections have become a serious problem as they result in more than 11 million outpatient/ emergency room visits and 464,000 hospitalizations annually in the United States [6]. Furthermore, the treatment of S. aureus infections has been increasingly formidable by the prevalent appearance of antibiotic-resistant strains such as methicillin-resistant S. aureus (MRSA) [7]. Although intense research and multiple diverse therapeutic trials have been applied at present, there is still no effective therapy for S. aureus infections.

Interleukin (IL)-33 is a newly identified member of IL-1 family of cytokines that binds to the IL-33 receptor, formerly known as the orphan receptor ST2 of the IL-1 receptor family [8]. Like the members of IL-1 family IL-1α and IL-1β, IL-33 is synthesized as a 31-kDa precursor that is cleaved by caspase 1 to produce a mature cytokine. However, unlike IL-1 and IL-18, which mainly implicated in Th1-type immunity, IL-33 displays a broadly immunoregulatory role in activation of Th2-associated immune response [9], promotion of macrophage and neutrophil migration [10,11], and induction of degranulation and maturation in eosinophils and mast cells [12,13]. It is well known that IL-33 is mainly localized to the nucleus of cells with barrier function such as endothelial and epithelial cells, and is released as a new alarmin in cell necrosis but not apoptosis that can alert the immune system [14]. An increasing body of evidence has shown that IL-33 is involved in the development of infection-related inflammation induced by pathogens, such as Pseudomonas aeruginosa, Pneumocystis murina and Candida albicans [15–17]. Nevertheless, the role of IL-33 in S. aureus-infected wound repair has not been investigated.

We have recently demonstrated that the expression of IL-33 is promptly enhanced in mucosal barrier damage such as in skin (a manuscript accepted for publication), lung and intestinal tissues [18,19], and the biological activity of IL-33 is primarily implicated in regulation of inflammatory response. In this study, we further explore the function of IL-33 in a mouse model of S. aureus-infected wound healing. We find that IL-33 messenger RNA and protein are rapidly increased during the tissue injury accompanied by MRSA infection.
Meanwhile, exogenous IL-33 administration can obviously accelerate wound healing by augmenting reepithelialization and extracellular matrix (ECM) deposition in a MRSA-infected incision model. Furthermore, the beneficial effect of IL-33 is closely related to promotion of the expression of CXCR2 and infiltration of neutrophils during early wound healing process.

2. Materials and methods

2.1. S. aureus strain

Meticillin-resistant S. aureus (MRSA) ATCC43300 was obtained from the American Type Culture Collection (ATCC). MRSA was inoculated in 10 μL of tryptic soy broth (Difco, Detroit, MI, USA) at 37 °C overnight and subcultured 1:100 in fresh tryptic soy broth; a log-phase culture was grown to an optical density of 0.3 at 650 nm (approximately 10^9 CFU/mL), washed three times with phosphate-buffered saline (PBS), and diluted in PBS.

2.2. Animals and IL-33

Male C57BL/6 mice (SPF grade) aged 8–10 weeks were purchased from the Center of Experimental Animals of Guangdong Province, and bred at an animal facility under pathogen-free conditions. All studies involving mice were approved by the Guangdong Pharmaceutical University Animal Care and Use Committee. Mouse IL-33 protein was prepared in our laboratory as described before [19].

2.3. Skin wound infection and IL-33 treatment

Infectious skin wounds caused by S. aureus were prepared as described previously [20]. Briefly, mice were anesthetized with pentobarbital (i.p., 50 mg/kg weight). Back skin hair was shaved, and then antisепtically prepared with 10% w/v povidone-iodine and 70% ethanol. A circular full thickness wound (5 mm in diameter) was made using a skin biopsy punch (Robbins instruments Inc., Chatham, NJ, USA). Immediately after skin wounding, mice were inoculated with 10^6 of 1 × 10^6 CFU MRSA at sites of skin wound. Murine recombinant IL-33 (10 μg/mouse) or vehicle (PBS) was injected i.p. once a day from day 5 to 8, starting immediately after wounding preparation to evaluate the wound-healing process. Photographs of the wounds were taken daily to follow gross visual wound healing as assessed by the area of the wound uncovered by the migrating epidermis. The rate of wound closure was expressed as a percentage of wound area relative to the original wound area.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Skin tissues were collected and homogenized in 500 μL of cold PBS supplemented with protease inhibitors (1 mmol/L phenylmethylsulfonyl fluoride, 1 μmol/L leupeptin, and 0.3 μmol/L aprotinin) using a Dounce homogenizer. Samples were then sonicated and centrifuged at 10,000 × g, and the resulting supernatant was stored at −80 °C. The level of IL-33 protein was measured using enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA, USA), according to the manufacturer's directions.

2.5. Histological processing of skin infection model

At the indicated intervals after S. aureus inoculation, skin lesion tissues were excised and fixed in 4% formaldehyde buffered with PBS (pH 7.2), and then embedded with paraffin. Five-micrometer thick sections were stained with HE or Masson's trichrome to observe the morphology or collagen deposition, and examined using light microscopy. Sections stained by Masson's trichrome were scored for the intensity and area of blue staining on a blinded 1–5 scale [21]. The CFU of MRSA in wound tissue was determined as described previously [20]. Briefly, at day 5 after MRSA inoculation, skin lesion tissues were homogenized in sterile PBS and plated on tryptic soy agar. After incubation at 37 °C overnight, the number of CFU of MRSA was quantified.

2.6. Immunohistochemistry

A double-color immunofluorescence analysis was conducted to determine C1d1b and Gr-1 positive cells as described previously [22]. Paraffin-embedded tissues were sectioned at 5 μm thickness, deparaffinized, and quenched with 3% hydrogen peroxide for 10 min. The sections were further incubated with PBS containing 1% normal serum corresponding to the secondary Ab and 1% bovine serum albumin (BSA) to reduce nonspecific reactions. The sections were stained with anti-C1d1b (clone M1/70; BD Pharmingen, San Jose, CA, USA) and anti-Gr-1 mAb (clone RB6-8C5; BD Pharmingen) at a concentration of 1 μg/mL at 4 °C overnight, and then with fluorescein-conjugated secondary antibodies.

2.7. Flow cytometry

Murine bone marrow cells and blood neutrophils were isolated and incubated with Fc Block (clone 93; Biolegend, San Diego, CA, USA) for 30 min, followed by a washing step with PBS containing 0.5% BSA. 1 × 10^6 cells were then incubated with phycocerythrin (PE)-labeled anti-C1d1b (clone M1/70; Biolegend), fluorescein isothiocyanate (FITC)-labeled anti-Gr-1 mAbs (clone RB6-8C5; Biolegend) and Alexa Fluor® 647-labeled anti-CXCR2 mAbs (clone TG11/CXCR2; Biolegend) for 30 min in the dark. After a washing step, the cells were acquired on a FACScalibur flow cytometer (BD Biosciences, San Jose, CA, USA), and the data were analyzed with the CELLQUEST v3.3 software as instructed.

2.8. Neutrophil isolation and in vitro chemotaxis assay

Neutrophils from peripheral blood were isolated by Percoll density gradient, as previously described [23]. Chemotaxis was performed in a 48-well microchamber (Neuroprobe, Gaithersburg, MD, USA) using a 5-μm pore polycarbonate membrane. Neutrophils (1 × 10^6 cells/mL) were allowed to migrate toward CXCCL2 (30 ng/mL) or medium alone at 37 °C for 1 h. The membrane was removed, fixed, and stained. Neutrophils that migrated through the membrane were examined by light microscopy.

2.9. Quantitative real-time PCR (qPCR)

Three micrograms of total RNA, extracted from uninjured and injured skin samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase I, was reverse-transcribed at 42 °C for 1 h in 20 μL reaction mixture containing mouse Moloney leukemia virus reverse transcriptase with oligo (dT) primers (Invitrogen). The resultant cDNAs were amplified together with SYBR Green qPCR kit (Invitrogen) using specific sets of primers as follows: IL-33 (forward 5′-CCTGCTTCCTCCTGAGTACATACA-3′, reverse 5′-CACGTGTTGACGCTTCCCTT-3′), KC (forward 5′-AGCTGCACATCAA-3′, reverse 5′-GT-3′), PTX (forward 5′-CACGTTGGAGATTGCTGCTG-3′, reverse 5′-CTTCTTCCCATCCACACC-3′), IL-1β (forward 5′-CTGCGCGAGTTGAAAGACAG-3′, reverse 5′-CTTTCTCCCATCCACACC-3′), KC (forward 5′-CACGTTGGAGATTGCTGCTG-3′, reverse 5′-CTTCTTCCCATCCACACC-3′), PTX (forward 5′-CACGTTGGAGATTGCTGCTG-3′, reverse 5′-CTTCTTCCCATCCACACC-3′), IL-1β (forward 5′-CTGCGCGAGTTGAAAGACAG-3′, reverse 5′-CTTTCTCCCATCCACACC-3′), KC (forward 5′-CACGTTGGAGATTGCTGCTG-3′, reverse 5′-CTTCTTCCCATCCACACC-3′), IL-1β (forward 5′-CTGCGCGAGTTGAAAGACAG-3′, reverse 5′-CTTTCTCCCATCCACACC-3′), KC (forward 5′-CACGTTGGAGATTGCTGCTG-3′, reverse 5′-CTTCTTCCCATCCACACC-3′), and GAPDH (forward 5′-TTCACCCAGAGAACGACT-3′, reverse 5′-GGCATGGACTGTGGTCATGA-3′), which were performed with an ABI PRISM® 7000 Sequence Detector Systems (Applied Biosystems, Foster City, CA, USA), and expression values were normalized to the housekeeping gene GAPDH using the comparative threshold cycle (CT) method.

Please cite this article as: Yin H, et al, IL-33 promotes Staphylococcus aureus-infected wound healing in mice, Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.07.008
2.10. Statistical analysis

Results are expressed as mean ± SEM. Statistical differences between groups were determined by the Student’s t test or one way ANOVA. Significance was claimed for $P < 0.05$.

3. Results

3.1. IL-33 expression is upregulated in MRSA-infected skin wounds

To investigate the local production of IL-33 during S. aureus infections, we used a previously established murine model of S. aureus-induced wound infection. C57BL/6 mice were inoculated with $1 \times 10^{8}$ CFU MRSA at the site of skin wound. As shown in Fig. 1A, mRNA level of IL-33 in wound tissue increased significantly at day 1 after MRSA infection, and remained elevated until 7 days after induction of infection. ELISA analysis further confirmed IL-33 temporal upregulation in response to MRSA infection at the protein level (Fig. 1B). Thus, these observations indicate that IL-33 signals may be implicated in MRSA-caused skin wound infection.

3.2. IL-33 accelerates infectious skin wound healing

As IL-33 levels were increased in S. aureus infection of skin wounds, we next identify the effect of IL-33 upregulation during the wound healing process. For this purpose, murine recombinant IL-33 (1.0 μg/day) was administered i.p. to mice subjected to incisional skin wounds with S. aureus infection once a day from day 0 to 5. As shown in Fig. 2A and B, wound closure was accelerated in IL-33-treated mice compared to wounds treated with vehicle. The ratio of wound area decreased, at day 3, by 94.6 ± 2.5% in IL-33-treated mice and by 103.6 ± 2.7% in vehicle-treated mice; the percentage of wound area was 69 ± 2.9 vs 86 ± 2.9% at day 5 and 32 ± 2.5 vs 55 ± 2.3% at day 7. This observation strongly suggests the essential role of IL-33 in wound healing process. For this purpose, murine recombinant IL-33 was administered i.p. to mice subjected to incisional skin wounds with S. aureus infection once a day from day 0 to 5. As shown in Fig. 2A and B, wound closure was accelerated in IL-33-treated mice compared to wounds treated with vehicle. The ratio of wound area decreased, at day 3, by 94.6 ± 2.5% in IL-33-treated mice and by 103.6 ± 2.7% in vehicle-treated mice; the percentage of wound area was 69 ± 2.9 vs 86 ± 2.9% at day 5 and 32 ± 2.5 vs 55 ± 2.3% at day 7. This observation strongly suggests the essential role of IL-33 in wound healing.

3.3. IL-33 abrogates wound infection caused by MRSA

The efficacy of IL-33 in antimicrobial infection in mice was further explored by examining the inflammatory cells infiltration and bacterial burden in these infectious skin tissues. Five days after infection, histological examinations revealed that wounds of IL-33-treated infected mice showed intense inflammatory infiltrate than did the untreated wounds at 5 days post-wounding after MRSA infection (Fig. 3A). Neutrophil infiltration was then determined by immunohistochemical assay. Consistent with the histological analysis, mice that received IL-33 treatment showed the higher population of CD11b and Gr-1 (neutrophil marker) double positive cells at 5 days after MRSA challenge compared to untreated mice (Fig. 3B and C). Meantime, the wound tissue was excised, homogenized and plated on tryptic soy agar. MRSA-infected IL-33-treated wounds showed significantly lower bacterial burden than did the untreated control wounds (Fig. 3D). Therefore, IL-33 treatment may have increased early inflammatory cell infiltration and accordingly enhanced bacterial clearance in infectious skin wound.

3.4. IL-33 promotes neutrophil proliferation and recruitment in MRSA-infected wounds

Successful clearance of bacterial infection depends on efficient neutrophil proliferation and trafficking into the infectious site [24]. The production of neutrophils in the bone marrow compartment was determined when mice challenged with MRSA. Neutrophils were identified by gating on Gr-1$^{\text{high}}$ and CD11b$^+$ fluorescence as detected by flow cytometry. As shown in Fig. 4A, IL-33 treatment significantly enhanced the number of neutrophils in the bone marrow in comparison with that of untreated mice. Meantime, real time PCR showed that IL-33-treated mice displayed an increased expression of chemokine KC in MRSA-infected tissues (Fig. 4B). It is well known that CXCR2 plays a crucial role in the activation and migration of neutrophils [25]. Consistent with these recent findings, MRSA infection lowered CXCR2 expression on neutrophils (Fig. 4C), and the reduction correlated with a lower chemotactic response to CXCL2 (Fig. 4D). Surprisingly, IL-33 treatment prevented the downregulation of CXCR2 on neutrophils caused by MRSA (Fig. 4C and D). Hence, IL-33-mediated protection is involved in augmenting the development and chemotaxis of neutrophils in infectious skin wound.

3.5. IL-33 improves extracellular matrix (ECM) production in MRSA-infected wounds

To determine the effect of IL-33 on ECM production during MRSA-infected wound healing, we examined skin samples histologically by Masson’s trichrome staining and quantified ECM-associated gene expression. Masson’s trichrome selectively colors collagens blue, and representative sections of treatment with IL-33 after MRSA infection are shown in Fig. 5A. A 0–5 scoring system was used to semiquantitatively assess total collagen staining at indicated time intervals (Fig. 5B). IL-33-treated mice had significantly increased the levels of total collagen than untreated control mice at day 5 post wounding. Meantime, ECM-related genes were also examined at day 5 post injury with 108 CFU MRSA at the site of skin wound. As shown in Fig. 1A, mRNA level of IL-33 in wound tissue increased significantly at day 1 after MRSA infection, and remained elevated until 7 days after induction of infection. ELISA analysis further confirmed IL-33 temporal upregulation in response to MRSA infection at the protein level (Fig. 1B). Thus, these observations indicate that IL-33 signals may be implicated in MRSA-caused skin wound infection.

![Fig. 1. IL-33 is rapidly produced at the site of MRSA surgical wound infection. Surgical wounds were established in C57BL/6 mice and challenged with MRSA. Wound tissue was excised at defined time points and the expression of IL-33 mRNA and protein was measured by real-time PCR and ELISA. (A) The levels of IL-33 mRNA are presented normalized to GAPDH expression. (B) The levels of IL-33 protein expressed as pictogram of protein in the total wound homogenate. Data are presented as the mean ± SEM (n = 6 in each group). *, P < 0.05 vs uninjured skin of C57BL/6 mice.](http://dx.doi.org/10.1016/j.intimp.2013.07.008)
and real time PCR showed that IL-33–treated mice exhibited substantially increased expression of related components of the ECM such as fibronectin and collagen IIIa, in comparison with that of untreated mice (Fig. 5C). Thereby, improved S. aureus-infected wound repair in IL-33-treated mice is consistent with a promotion in the expression of ECM-associated genes during wound healing.

Fig. 2. IL-33 promotes cutaneous wound closure after MRSA infection. (A) The infectious wound sites were photographed at defined time points. day 0 picture was taken immediately after the injury. Representative results from 10 individual animals in each group are shown. (B) Graphical summary of changes in wound area, expressed relative to the initial size of the wound at day 0. Data are presented as the mean ± SEM (n = 6 in each group).* P < 0.05 vs untreated controls.

Fig. 3. IL-33 ameliorates cutaneous wound infection caused by MRSA. Five days following MRSA challenge, the histopathological tissue damage and wound bacterial burden were determined. (A) Skin wound sections from IL-33–treated or -untreated group were stained with hematoxylin & eosin (100×). Wound margins indicated by arrows. (B) A double-color immunofluorescence analysis was performed using anti-CD11b (PE) and anti-Gr-1 mAb (FITC) and observed under a fluorescence microscope (× 100). Red and green fluorescence correspond to CD11b-positive cells and Gr-1-positive cells, respectively. Yellow indicates CD11b and Gr-1 double positive neutrophils. Representative results from three independent experiments are shown. (C) Mean number of CD11b+Gr-1+ cells per high-powered field. (D) Infected skin tissue was removed from mice and bacterial burden (CFU; colony forming unit) was determined. Data are presented as the mean ± SEM (n = 6 in each group).* P < 0.05 vs untreated controls. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
IL-33 promotes Staphylococcus aureus-infected wound healing in mice. Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.07.008
burst and chemotaxis are mediated mainly by CXCR1 and CXCR2, respectively. Several studies have reported that IL-33, through upregulating CXCR2 expression, plays a critical role in neutrophil migration and improved bacterial clearance [11]. In addition, using incisional skin wounds, Devalaraja et al. demonstrated that neutrophil recruitment in CXCR2−/− mice was considerably reduced throughout the wound healing process and subsequently delayed wound repair [35].

In consistent with these studies, we found that exogenous IL-33 administration obviously contributed to the expression of CXCR2 and chemotaxis of neutrophils. Furthermore, IL-33 treatment in a mouse model of MRSA-caused wound infection promptly accelerated neutrophil infiltration into wound tissues. These findings imply that migration of neutrophils in early wound infection promoted by IL-33 is crucial for bacterial phagocytosis and wound healing.

In summary, the presented data reveal that IL-33 plays a substantial role in MRSA-caused skin wound infection. Treatment with IL-33 significantly accelerates the proliferation of neutrophils and CXCR2 expression, which is involved in early augmented neutrophil trafficking into infectious sites for bactericidal activity. In addition, the beneficial effect of IL-33 is also related to enhancement of collagen deposition and reepithelialization during wound repair. Therefore, these results suggest that IL-33 may provide a profound effect in maintenance of cutaneous homeostasis and acceleration of MRSA-infected wound healing.

Acknowledgments

The project was supported by Guangdong Natural Science Foundation (Grant S2012010009409), the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry [No (2011)1139] and National Natural Science Foundation of China [21206020].

References


Please cite this article as: Yin H, et al, IL-33 promotes Staphylococcus aureus-infected wound healing in mice, Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.07.008


