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Noninvasive nuclear factor-κB bioluminescence imaging for the assessment of host–biomaterial interaction in transgenic mice

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Abstract

The inflammatory response is a key component in the biocompatibility of biomaterials. Among the factors that control the development of inflammation is a critical molecule nuclear factor-κB (NF-κB). Therefore, the aim of this study was to assess the feasibility of noninvasive whole-body real-time imaging for the evaluation of host–biomaterial interaction in the NF-κB transgenic mice. Transgenic mice, carrying the luciferase gene under the control of NF-κB, were constructed. *In vivo* bioluminescence imaging showed that the constitutive and induced NF-κB activities of transgenic mice were detected in most of the lymphoid tissues, demonstrating that NF-κB-driven luminescence reflected the inflammatory response *in vivo*. By the implantation of genipin-cross-linked gelatin conduit (GGC) and bacterial endotoxin-immersed GGC in the dorsal region, we detected a strong and specific luminescent signal from the tissue around the bacterial endotoxin-immersed GGC implant. Histological and immunohistochemical analysis also demonstrated that inflammation, characterized by the infiltration of immune cells, the accumulation of fluid, and the activation of NF-κB, was evoked around the same region. The correlation between the bioluminescence imaging and histological changes indicated that noninvasive imaging technique could be used to monitor the real-time inflammation in the implanted mice.

Keywords: Inflammation; Bioluminescence imaging; Genipin-cross-linked gelatin conduit; Nuclear factor-κB; Transgenic mice

1. Introduction

One of the most important host–biomaterial interactions is the putative immune response evoked by the implantation of biomaterials [1]. Inflammation is the first innate response of immune system to infection or irritation and is characterized by redness (*rubor*), heat (*calor*), swelling (*tumor*), pain (*dolor*), and dysfunction of the organs involved (*functio laesa*). The development of inflammatory response is controlled by various cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-12 (IL-12), tumor necrosis factor-α (TNF-α), and interferon-γ, which are secreted by phagocytes [2]. The production of cytokine is further controlled by the transcription factor, nuclear factor-κB (NF-κB) [3].

NF-κB is a nuclear transcription factor that consists of heterodimers of RelA (p65), c-Rel, RelB, p50, and p52. NF-κB activity is induced by bacteria, viruses, necrotic cell products, and inflammatory cytokines. When stimulated, NF-κB binds to the NF-κB-responsive element present in the promoter of inflammatory genes, leading to the induction of gene expression. Accordingly, NF-κB is a critical molecule involved in the regulation of inflammatory cytokine production and inflammation [4–6].

Traditionally, the inflammatory response evoked by the implantation of biomaterials is evaluated by histological changes. However, the sacrifices of mice are necessary for obtaining the tissues in this method. Reverse transcription-polymerase chain reaction (RT-PCR) has also been applied to investigate the contents of inflammatory cytokines

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involved in the host–biomaterial interaction [7–9]. These methods take samples from living animals; however, preparations of samples for detection are very expensive and time-consuming. Moreover, all of these methods cannot reflect the real-time inflammatory status in animals. Noninvasive imaging techniques have been used to directly visualize and quantify molecular, cellular, and tissue level interactions with the biomaterials *in vitro* and *in vivo*. For examples, micro-computed tomography (micro-CT) has been used to analyze mineral formation *in vitro* and to assess the bond growth and scaffold–bond interaction *in vivo* [10–13]. Fourier transform infrared imaging has been applied in the analysis of bond and cartilage in healthy and diseased tissues, and the characterization of tissue-engineered bone and cartilage [14]. Magnetic resonance image (MRI) has been employed to monitor the degradation process of bioresorbable polymeric implants *in vivo* [15]. Recently, bioluminescence imaging, relying upon the ability to detect and quantify the light originating from luciferase-labeled cells and animals, has been used to monitor the performance of scaffold–stem cell combination implanted in living animals and the growth of cells on biomaterials [16,17]. In this study, we constructed the transgenic mice carrying the luciferase gene driven by NF-κB-responsive elements. Genipin-cross-linked gelatin conduit (GGC) was implanted subcutaneously in the dorsal region of transgenic mice and the inflammatory response was monitored by noninvasive bioluminescent imaging (Fig. 1(A)). Results presented here demonstrated that noninvasive real-time imaging system can be used to assess host–biomaterial interactions in living animals.

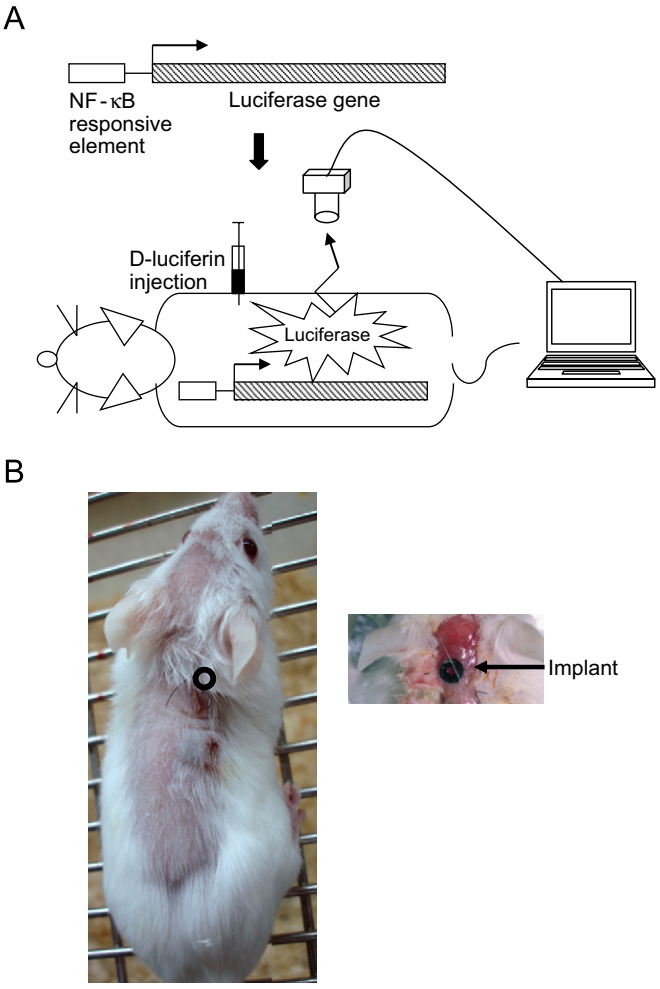

Fig. 1. (A) Construction and *in vivo* imaging of transgenic mice. The schematic diagram of NF-κB-luc plasmid DNA is shown on the top. The expression of luciferase was driven by NF-κB-responsive element (5′-GGGACTTTCC-3′). NF-κB-luc plasmid DNA was used to generate transgenic mice using microinjection technique. Imaging of transgenic mice was performed with the IVIS imaging system on anesthetized mice following intraperitoneal injection of D-luciferin. Immediately afterward the mice were placed in a ventral recumbent position in the chamber and imaged with the camera set. (B) Implantation of GGC in transgenic mice. Left diagram shows the location of GGC implant. Gross observation of GGC implant is shown at the right.

2. Materials and methods

2.1. Materials

GGC (1.96 mm in diameter, 1.5 mm in length) was prepared as described previously [18]. Lipopolysaccharide (LPS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO) and dissolved in water and phosphate-buffered saline (PBS) (137 mM NaCl, 1.4 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 2.7 mM KCl, pH 7.2), respectively. D-Luciferin was purchased from Xenogen (Hopkinton, MA) and dissolved in PBS at 15 mg/ml. Mouse monoclonal antibodies against luciferase and NF-κB were purchased from Santa Cruz (Santa Cruz, CA) and Chemicon (Temecula, CA), respectively.

2.2. Generation of transgenic mice

Plasmid DNA NF-κB-luc was constructed by positioning five NF-κB-responsive elements (5′-GGGACTTTCC-3′) upstream of a firefly luciferase cDNA (derived from pGL3-Basic, Promega, Madison, WI). NF-κB-luc construct was tested by transient and stable transfections in HepG2 cells. This same construct was linearized with *Bam*HI to generate a 2.7-kb fragment of NF-κB-luc transgene for generation of transgenic mice following pronuclear microinjection of FVB oocytes. Of 25 offspring (F_0), six tested positive for NF-κB-luc by PCR genotyping (primer-P 5′-AACTGCATAAGGCTATGAAGAGATACGCCC-3′ and primer-M 5′-TTAAAACCGGGAGGTAGATGAGATGTGACG-3′). All transgenic mice were crossed with wild-type F_1 mice to yield NF-κB-luc heterozygous mice with the FVB genetic background.

2.3. Cell culture and LPS treatment

Recombinant HepG2/NF-κB cells, which contained the luciferase gene driven by NF-κB-responsive element, were constructed previously [19]. HepG2/NF-κB cells were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum. For LPS treatment, cells were cultured in 96-well plates at 37 °C. After a 24-h incubation, cells were washed and starved with DMEM for an additional 24 h. Various amounts of LPS were then treated cells for 16 or 24 h.

2.4. Luciferase assay

HepG2/NF-κB cells were treated with LPS for 16 h. Luciferase assay was performed as described previously [20]. Relative NF-κB activity was calculated by dividing the relative luciferase unit (RLU) of treated cells by the RLU of untreated cells.