TACONIC

LPTA ANIMAL MODELS

LPTA Animal Model *Tnfα-luc Xen*

Information

Mouse Strain:	BALB/C-Tg(Tnfα-luc) -Xen
Common Name:	*Tnfα-luc*
Genotype:	Hemizygous
Background Strain:	BALB/C
Coat Color:	Albino

Key Applications

- **Inflammation**
  - Arthritis, Inflammatory bowel disease
- **Cancer**
  - Apoptosis
- **Sepsis**

Introduction:

TNFα is one of the earliest cytokines produced by activated monocyte-macrophages and plays a pivotal role in the pathogenesis of inflammation, septic shock, and tissue injury. TNFα responses in target cells are mediated by TNFα receptors. Although TNFα is produced predominantly in monocyte-macrophages, it is expressed by numerous other cells and can be activated by a wide range of stimuli. The principal source of serum TNFα during endotoxemia is the liver. Several regulatory sequences are found upstream of the TNF gene, including NFκB sites, NF-AT-binding sites, cAMP responsive elements for ATF- 2/JUN, SP-1 sites, AP-1 and AP-2 sites for FOS/JUN, and EGR-1 binding sites. These regulatory sequences can bind to various transcriptional factors such as NFκB, FOS/JUN, and NF-AT. TNFα expression is inhibited by p38 MAP kinase inhibitors.[1]

Description of Model:

The light producing animal model (LPTA®) is BALB/C-Tg(Tnfα-luc)-Xen, commonly called Tnfα-luc, carries a transgene containing a 2.5 kb upstream region of the murine TNFα gene, the native intron of the TNFα gene, modified firefly luciferase cDNA

(Promega pGL-3), and a 1 kb downstream region of the TNFα gene containing the TTATTTAT repeat, which is important for TNFα gene regulation. The reporter is inducible by LPS. Expression of the reporter was higher in LPS-treated mice than control mice, and highest in kidney, lung, intestine, heart, and fat. The model provides for the rapid study of transcriptional regulation of the TNFα gene and indications of the efficacy of therapeutics that are targeted on modulating TNFα expression.

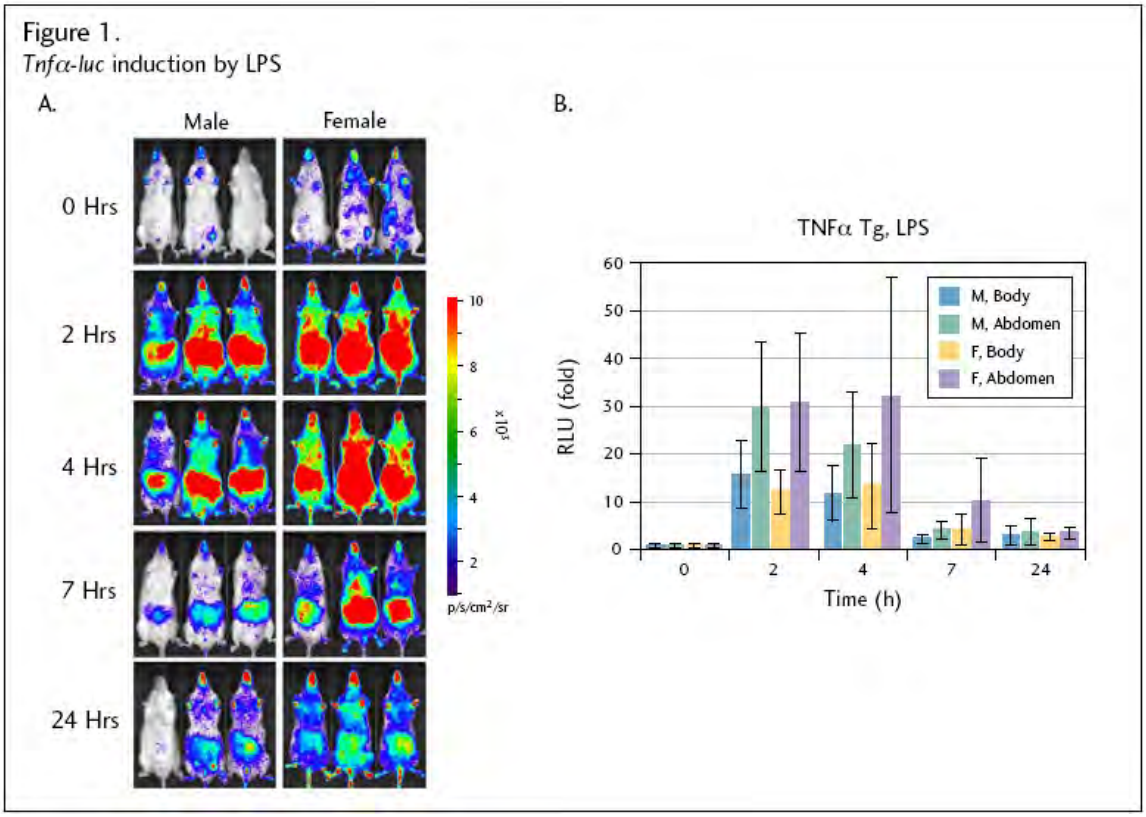

Figure 1. The mice (n=3) were imaged at T= 0 (pretreatment) and 2, 4, 7 and 24 hours following intraperitoneal injection of LPS (1mg/kg) (A). Photons/sec quantified from liver region and whole body. The data (B) represent mean fold of induction.

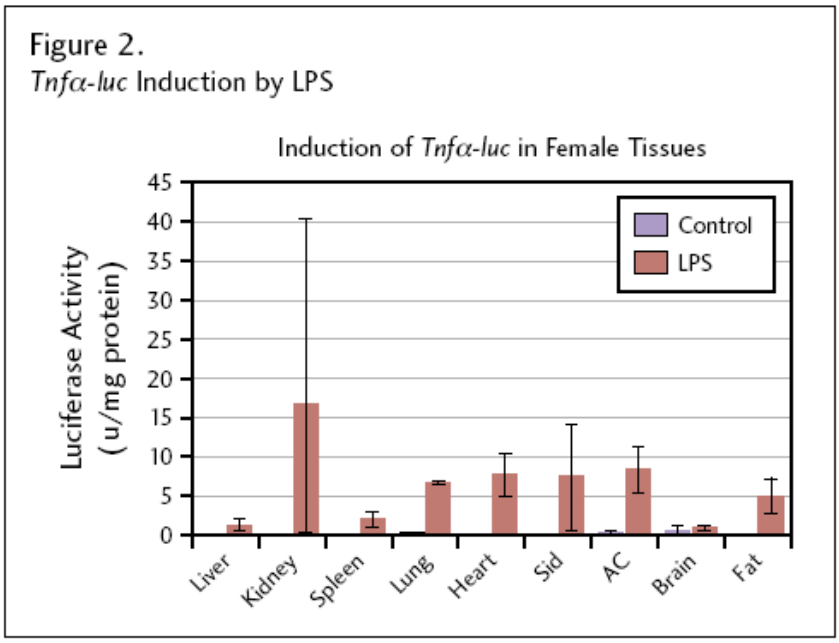

Figure 2. Female mice (n=3) were sacrificed at 3 hours following intraperitoneal injection of LPS (1mg/kg) or saline (control). Organs were harvested and processed for both luciferase and protein measurement. Two sections of the intestine were analyzed, Sid (duodenum) and Ac (ascending colon). The data represent luciferase activity as units/mg protein.

Origin

The background strain is BALB/C. This transgenic line was created by microinjection method using BALB/C donor fertilized eggs.

Applications

The Tnfα-luc LPTA® animal model is useful in studying sepsis, arthritis, inflammatory bowel disease, apoptosis, TNFα gene regulation, and the treatment of TNFα mediated inflammatory diseases.

Example Phenotype

The coat color of the LPTA® animal model is albino. Tnfα-luc mice have been observed to have litters of 8–12 pups. At ≥ 6 weeks of age, adult mice weights were appropriate: 20–30g. The Tnfα-luc transgene is induced with LPS. Treatment with LPS triggered a 30-fold luciferase induction in male and female abdominal region at the peak of 2–4 hours (Figure 1, page 1). LPS-treated mice exhibit higher levels of luciferase expression than control mice in all tissues. Luciferase activity in LPS treated mice was highest in kidney, lung, intestine, heart, and fat (Figure 2).

Genotype

The transgenic line is hemizygous. The presence of the Tnfα-luc transgene was determined from mice using PCR. The 1 kb PCR product from the sequence internal to the luciferase gene was amplified using forward primer:

(5´-TGGATTCTAAAACGGATTACCAGGG -3´)
and reverse primer:
(5´-CCAAAACAACAACGGCGGC-3´).

PCR conditions: 97ºC 5:00 min; 94.5ºC 0:40 min; 58ºC 1:30 min; 72ºC 1:30 min for 35 cycles; 72ºC 10:00 min; hold at 4ºC until analysis.

Note: An NFκB-RE-luc transgenic mouse model was also developed by Dr. R. Blomhoff[4].

References

1. Wang H, Tracey KJ. Tumor necrosis factor, interleukin-6, macrophage migration inhibitory factor, and macrophage inflammatory protein-1 in inflammation. In: Gallin JI, Snyderman R, eds. Inflammation: Basic Principles and Clinical Correlates. 8th ed. Philadephia, PA: Lippincott Williams & Wilkins; 1999:471-486.

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