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Yamamura et al.

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(54) **INFLAMMATION REPORTER SYSTEM**

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(57) **ABSTRACT**

The present invention provides a method for detection of an inflammatory reaction, which comprises using a transformant or transgenic non-human animal transfected with a vector comprising a promoter for a gene encoding an inflammatory cytokine, a gene encoding a reporter protein, a gene encoding the inflammatory cytokine, and a gene encoding a proteolytic signal sequence to thereby detect an inflammatory reaction induced upon inflammatory stimulation in the transformant or in the transgenic non-human animal.

11 Claims, 8 Drawing Sheets
(4 of 8 Drawing Sheet(s) Filed in Color)
Specification includes a Sequence Listing.

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Matsushima et al., "Intravital Imaging of IL-1β Production in Skin," Journal of Investigative Dermatology (2010), vol. 130, pp. 1571-1580.

Oikawa et al., "A transgenic mouse model for monitoring oxidative stress," Scientific Reports (Jan. 19, 2012), vol. 2, No. 229, pp. 1-8.

Iwawaki et al., "Transgenic mouse model for imaging of interleukin-1β-related inflammation in vivo," Scientific Reports, vol. 5, No. 1, Dec. 2015 (Published Nov. 24, 2015), pp. 1-10, XP055428772.

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Figure 1

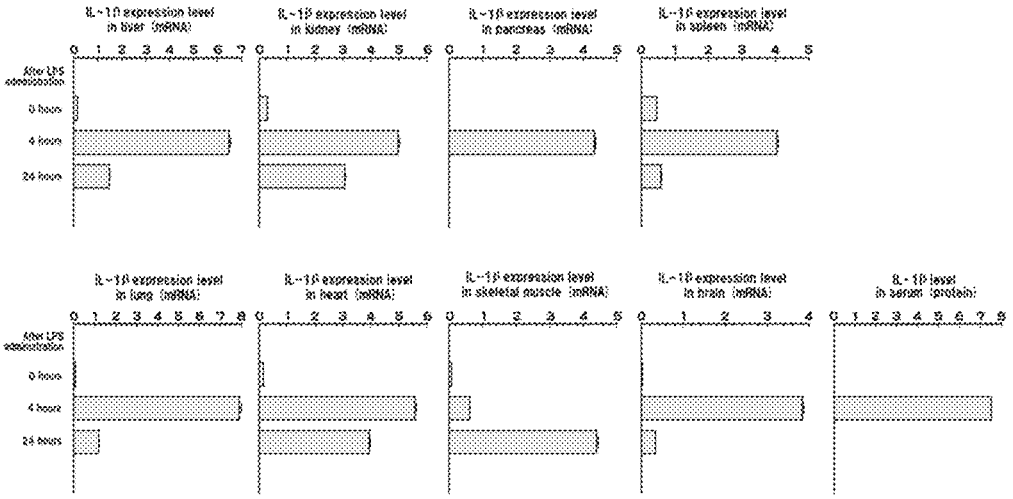

IL-1β expression level in liver (mRNA)
IL-1β expression level in kidney (mRNA)
IL-1β expression level in pancreas (mRNA)
IL-1β expression level in spleen (mRNA)
IL-1β expression level in lung (mRNA)
IL-1β expression level in heart (mRNA)
IL-1β expression level in skeletal muscle (mRNA)
IL-1β expression level in brain (mRNA)
IL-1β level in serum (protein)
After LPS administration
0 hours
4 hours
24 hours

Figure 2

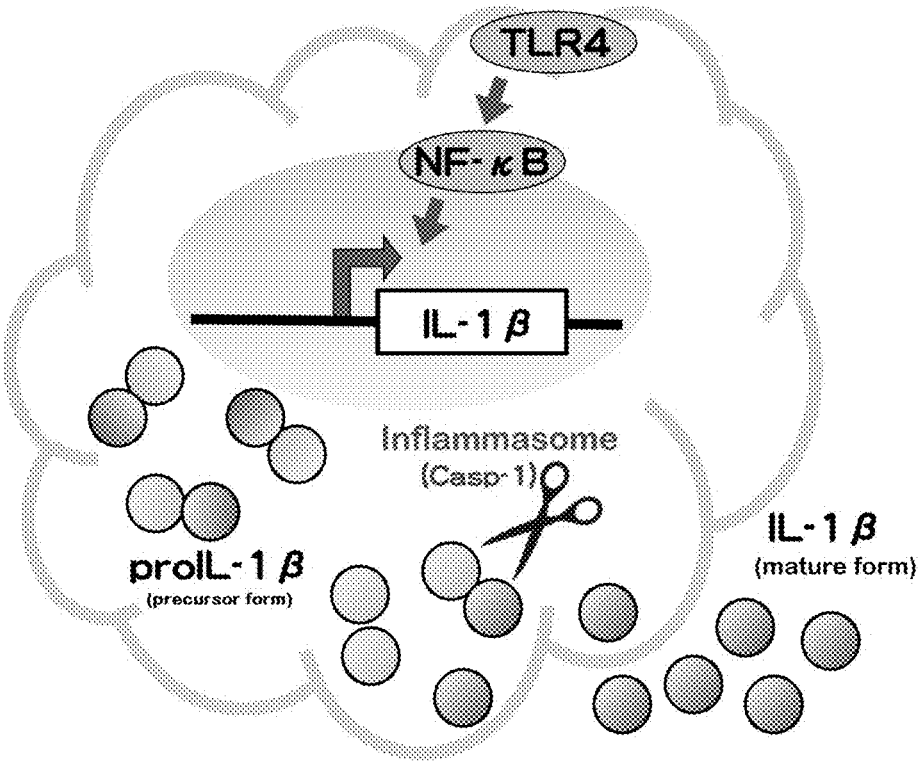

TLR4
NF-κB
IL-1β
Inflammasome
(Casp-1)
proIL-1β
(precursor form)
IL-1β
(mature form)

Figure 3

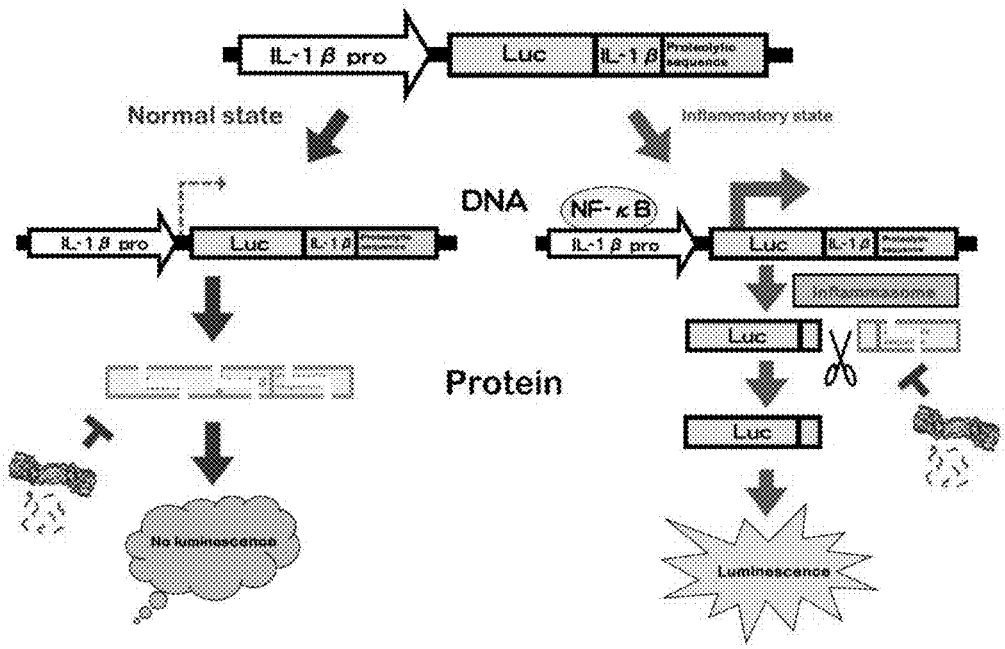

IL-1β pro
Luc
IL-1β
Normal state
Inflammatory state
DNA
NF-κB
IL-1β pro
Luc
IL-1β
Protein
Luc
Luc
No luminescence
Luminescence

Figure 4

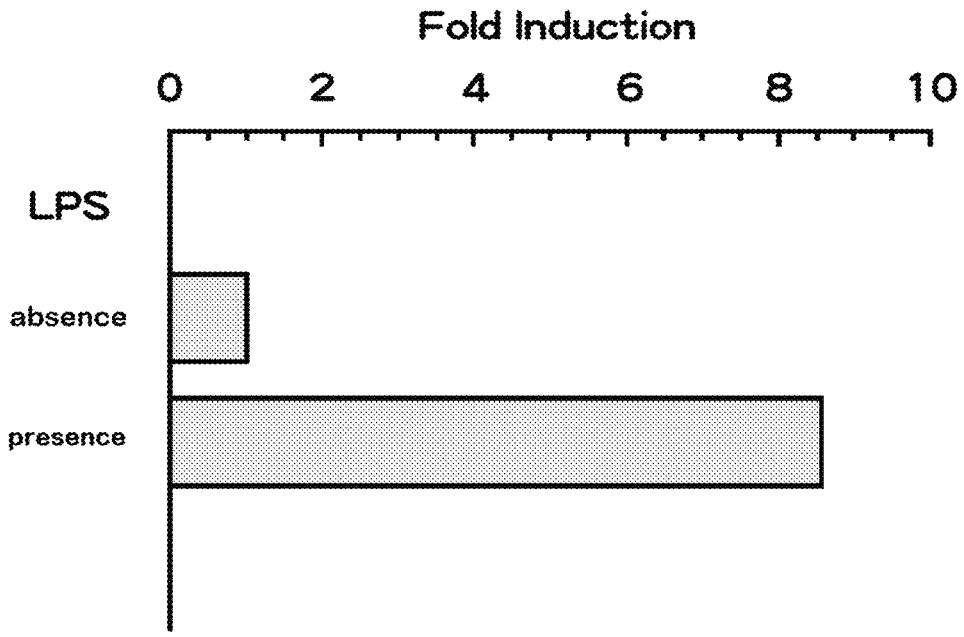


Figure 5

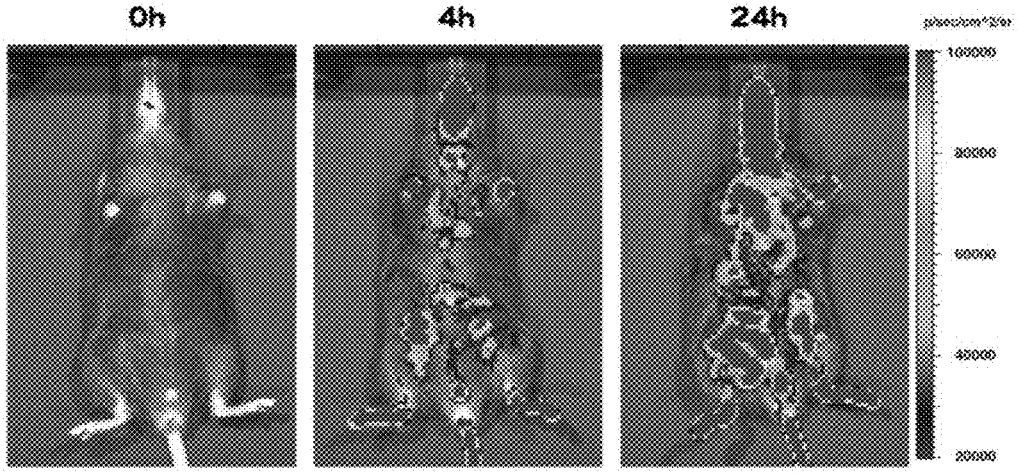


Figure 6

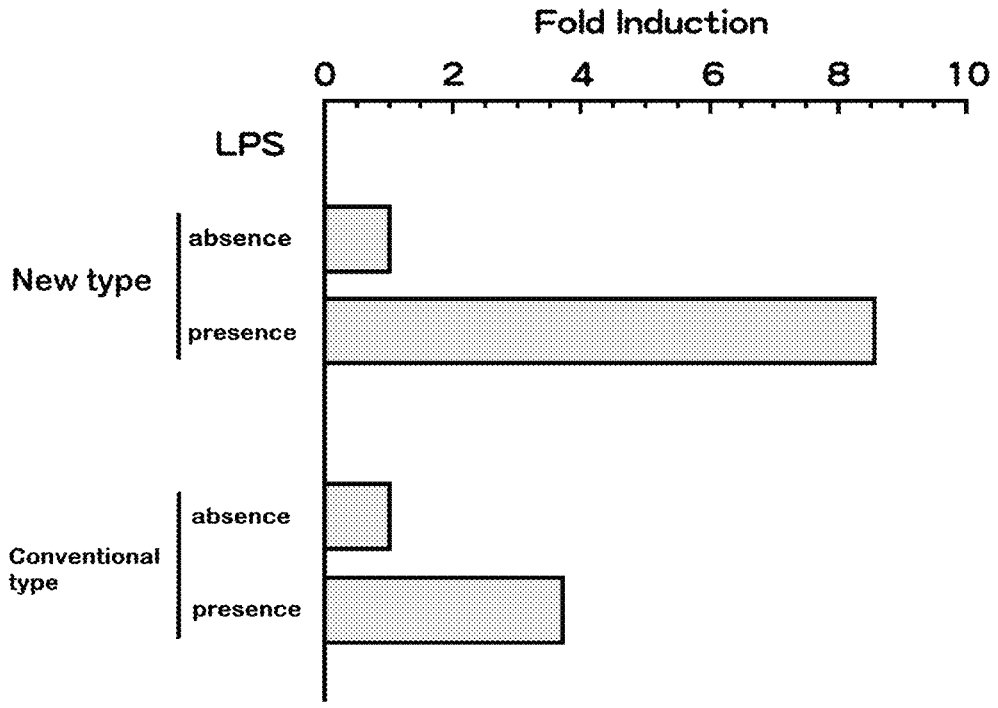

Fold Induction
0
2
4
6
8
10
LPS
New type
absence
presence
Conventional type
absence
presence

Figure 7

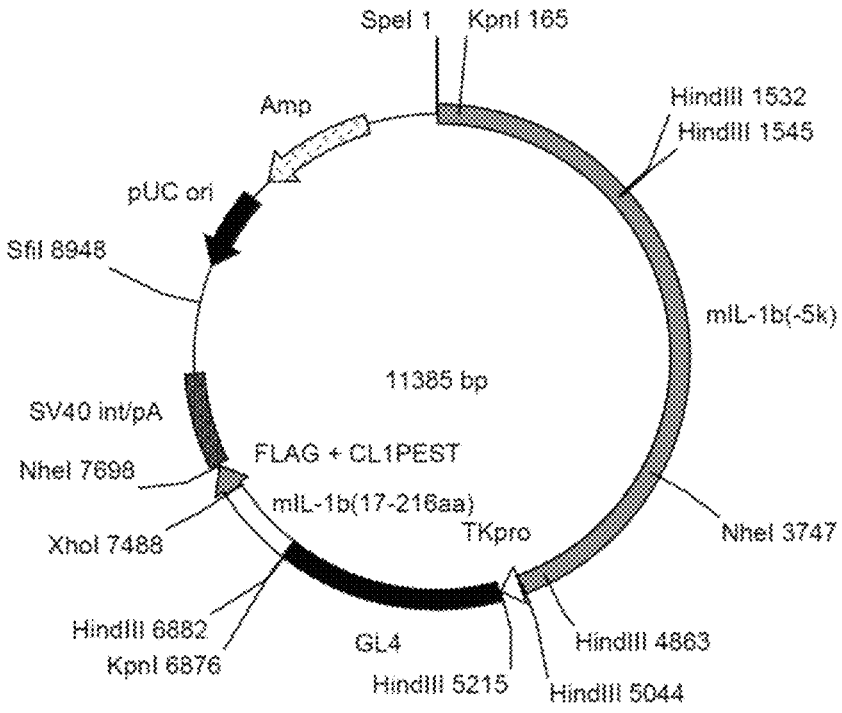
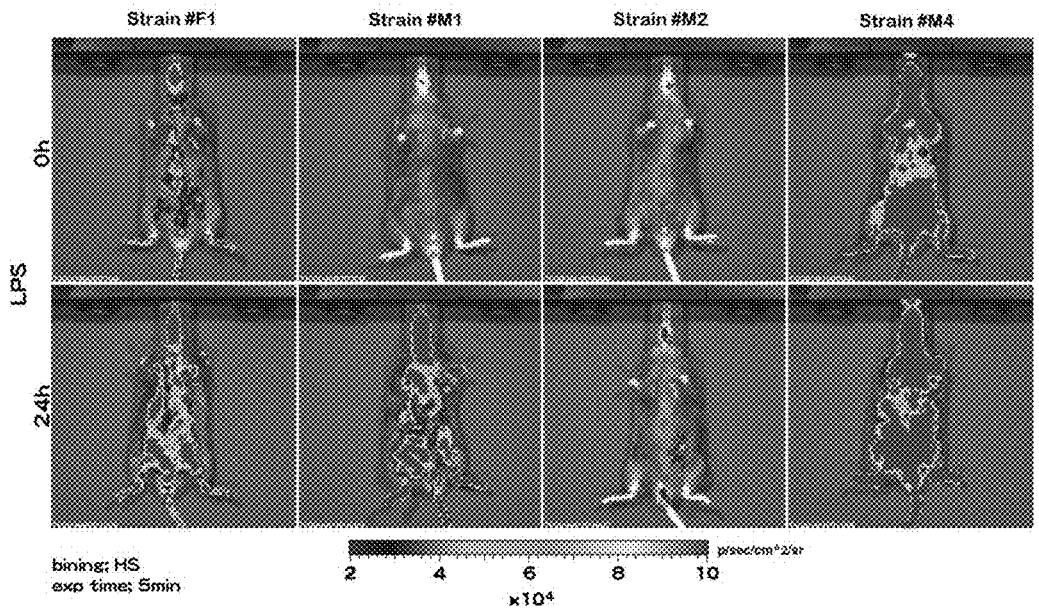

SpeI 1
KpnI 165
HindIII 1532
HindIII 1545
Amp
pUC ori
SfiI 8948
mIL-1b(-5k)
11385 bp
SV40 int/pA
FLAG + CL1PEST
NheI 7698
mIL-1b(17-216aa)
TKpro
XhoI 7488
NheI 3747
HindIII 6882
KpnI 6876
GL4
HindIII 5215
HindIII 4863
HindIII 5044

Figure 8


Strain #F1
Strain #M1
Strain #M2
Strain #M4
0h
24h
LPS
binning: HS
exp time: 5min
2
4
6
8
10
×10⁴
p/sec/cm²/sr

INFLAMMATION REPORTER SYSTEM

TECHNICAL FIELD

The present invention relates to a method for detection of an inflammatory reaction.

BACKGROUND ART

Inflammatory reaction is one of the body's responses deeply involved in the symptoms of many diseases, and is now an important subject of study in understanding their pathology and/or in developing therapeutic strategies against these diseases. For this reason, it is indispensable to develop a technique which allows detection of the actual conditions of inflammation.

A typical inflammatory reaction has been elucidated to occur through the following mechanism. Namely, once a source of infection (e.g., bacteria, viruses) has entered the body, the source of infection will be detected by cell surface receptors, followed by induction of cytokine secretion. The secreted cytokines will serve as guides to cause immunocytes (e.g., macrophages) to migrate to the infection site, whereby the source of infection will be eliminated. The increased activity of these immunocytes during elimination will result in flare, fervescence, pain and swelling which are characteristic of inflammation.

As a cytokine which is significantly involved in this inflammatory reaction and also receives attention as an inflammatory marker, interleukin-1 beta (IL-1β) has been known. IL-1β is not substantially secreted in the absence of inflammatory stimulation, but is known to be produced and secreted at a very high level in each tissue upon inflammatory stimulation (FIG. **1**).

IL-1β has been found to be strictly regulated by the following characteristic two-stage control. The gene expression of IL-1β is activated by transcription factor NF-κB induced during inflammatory reaction, and the activated IL-1β gene expression in turn promotes the production of precursor proIL-1β. Then, proIL-1β will be cleaved by caspase activated in inflammasomes and converted into secretable mature IL-1β (FIG. **2**).

Some reports have been issued about the monitoring of IL-1β gene expression in which luciferase or red fluorescent protein is used as a reporter molecule (Non-patent Documents 1 and 2). In these reports, it is shown that transgenic mice carrying a reporter molecule are prepared and in this inflammation model, reporter signals can be detected and also can be used for in vivo imaging analysis. However, this method relies only on transcriptional regulation, which is one factor contributing to a cascade of inflammatory reactions, and hence this method is insufficient to monitor physiological inflammatory reactions.

On the other hand, a reporter system regulated by inflammasomes has also been reported (Non-patent Document 3). In this report, a reporter molecule is design such that it is in an inactive state due to aggregation in the absence of inflammation, but it will be converted into a monomer form to exert its activity when inflammasomes become functional upon inflammatory stimulation. However, this system also relies only on inflammasomes and is therefore insufficient in sensitivity. Moreover, this system has not been verified as to whether it is functional in living mice.

Other attempts have also been made to induce protein expression by various internal or external stimuli (e.g., oxidative stress, endoplasmic reticulum stress) and to visualize the event taking place (Patent Documents 1 and 2, Non-patent Documents 4 and 5).

PRIOR ART DOCUMENTS

Patent Documents

Patent Document 1: WO2012/099279
Patent Document 2: Japanese Patent No. 4446057

Non-Patent Documents

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Non-patent Document 3: Bartok E. et al "iGLuc: a luciferase-based inflammasome and protease activity reporter" Nat. Methods., vol. 10, 147-154 (2013)
Non-patent Document 4: Scientific Reports 2012; 2:229. Epub 2012 Jan. 19.
Non-patent Document 5: Nature Medicine 10, 98102 (1 Jan. 2004)

SUMMARY OF THE INVENTION

Problem to be Solved by the Invention

The present invention aims to provide a technique which allows highly efficient and highly sensitive detection and measurement of a local inflammatory reaction particularly in a microregion in the living body. The present invention also aims to provide a gene vector which allows easy use of this detection method, as well as a transgenic mouse transfected with the gene vector of this reporter system, which allows further use in in vivo research and development.

Means to Solve the Problem

As a result of repeating extensive and intensive efforts to solve the problems stated above, the inventors of the present invention have found that based on the mechanism of inflammatory reaction which is regulated in two stages by the IL-1β gene and inflammasomes, a reporter system can be constructed which allows highly efficient and highly sensitive monitoring of physiological inflammatory reactions. Thus, the inventors of the present invention have constructed this monitoring method and a gene vector provided with this method, as well as a transgenic mouse, thereby completing the present invention.

Namely, the present invention is as follows.

(1) A vector comprising a promoter for a gene encoding an inflammatory cytokine, a gene encoding a reporter protein, a gene encoding the inflammatory cytokine, and a gene encoding a proteolytic signal sequence.

(2) The vector according to (1) above, wherein the inflammatory cytokine is interleukin 1β.

(3) The vector according to (1) or (2) above, wherein the reporter protein is luciferase.

(4) The vector according to any one of (1) to (3) above, wherein the gene encoding the inflammatory cytokine comprises a polynucleotide sequence encoding a peptide recognizable by caspase.

(5) A transformant comprising the vector according to any one of (1) to (4) above.

(6) A transgenic non-human animal transfected with the vector according to any one of (1) to (4) above.

(7) The transgenic non-human animal according to (6) above, wherein the non-human animal is a mouse.

(8) The transformant according to (5) above or the transgenic non-human animal according to (6) or (7) above, wherein the reporter protein is detected as a luminescence signal upon inflammatory stimulation.

(9) A method for detection of an inflammatory reaction, which comprises using the transformant according to (5) above or the transgenic non-human animal according to any one of (6) to (8) above to detect an inflammatory reaction induced upon inflammatory stimulation in the transformant or in the transgenic non-human animal.

(10) The method according to (9) above, wherein the gene encoding the inflammatory cytokine is expressed by transcription factor NF-κB induced during inflammatory reaction.

(11) The method according to (9) or (10) above, wherein the reporter protein is detected as a luminescence signal upon inflammatory stimulation.

(12) A method for screening of anti-inflammatory substances, which comprises bringing the transformant according to (5) above or the transgenic non-human animal according to any one of (6) to (8) above into contact with candidate substances under inflammatory stimulation to select an anti-inflammatory substance on the basis of the presence or absence of an inflammatory reaction serving as an indicator.

(13) A kit for detection of an inflammatory reaction or for screening of anti-inflammatory substances, which comprises the transformant according to (5) above or the transgenic non-human animal according to any one of (6) to (8) above.

Effects of the Invention

The present invention provides a reporter system which allows highly efficient and highly sensitive monitoring of inflammatory reactions. The system of the present invention allows visualization of inflammatory reactions and achieves extremely high sensitivity and efficiency.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one color drawing. Copies of this patent or patent application publication with color drawing will be provided by the USPTO upon request and payment of the necessary fee.

FIG. **1** shows changes in IL-1β levels after inflammatory stimulation with lipopolysaccharide (LPS). LPS used: SIGMA #L2654, concentration of use: 3 to 4 μg/g body weight

FIG. **2** shows the regulatory mechanism for IL-1β production and secretion.

FIG. **3** shows the construction of an inflammation reporter system based on IL-1β.

FIG. **4** shows reporter activity in RAW264 transiently transfected with a reporter gene. LPS used: SIGMA #L2654, concentration of use: 2 μg/ml

FIG. **5** shows signals emitted from LPS-stimulated inflammation reporter mice. LPS used: SIGMA #L2654, concentration of use: 3 to 4 μgig body weight

FIG. **6** shows reporter activity in RAW264 transiently transfected with a reporter gene. LPS used: SIGMA #L2654, concentration of use: 2 μg/ml, treatment time: 48 hours

FIG. **7** shows the construction of the vector of the present invention.

FIG. **8** shows the results compared for reporter signals before and after LPS stimulation among mouse strains.

DESCRIPTION OF EMBODIMENTS

The present invention will be described in more detail below.

1. Vector and Others as Well as Detection Method

The vector used in the present invention comprises a fusion gene composed of multiple genes ligated together, and is designed to express a fusion protein composed of a reporter molecule, a caspase recognition sequence and a proteolytic signal sequence under the control of a promoter for a gene encoding an inflammatory cytokine.

In the context of the present invention, the term "inflammatory cytokine" refers to a cytokine which is produced from helper T cells, monocytes, macrophages, neutrophils, dendritic cells or other cells upon activation with an antigen (e.g., bacteria) and which activates macrophages or other cells of the immune system, vascular endothelial cells or osteoclasts. Examples of such an inflammatory cytokine include IL-1β, IL-6, IL-8, IL-12, IL-13, IL-17, IL-18, tumor necrosis factor (TNF) and so on. Genes encoding inflammatory cytokines are expressed by transcription factor NF-κB induced during inflammatory reaction.

In the present invention, genes encoding these inflammatory cytokines or partial sequences thereof may be used. Information is known about genes encoding the above inflammatory cytokines and promoters for these genes. Partial sequences may be of any length as long as they ensure responsiveness to inflammation, and their length and region may be determined on the basis of enhanced expression in response to inflammation and/or processing in response to inflammation, etc.

IL-1β: Accession No. NM_008361.3
IL-6: Accession No. NM_031168.1
IL-8: Accession No. NM_009140.2
IL-12: Accession No. NM_001159424.1
IL-13: Accession No. NM_008355.3
IL-17: Accession No. NM_010552.3
IL-18: Accession No. NM_008360.1
TNF: Accession No. NM_001278601.1
IL-1β promoter: Accession No. NC_000068.7
IL-6 promoter: Accession No. NC_000071.6
IL-8 promoter: Accession No. NC_000071.6
IL-12 promoter: Accession No. NC_000069.6
IL-13 promoter: Accession No. NC_000077.6
IL-17 promoter: Accession No. NC_000067.6
IL-18 promoter: Accession No. NC_000075.6
TNF promoter: Accession No. NC_000083.6

For convenience of explanation, IL-1β is taken as an example herein.

A reporter gene is ligated downstream of a promoter for the IL-1β gene, and a gene construct comprising, for example, a IL-1β partial sequence and a proteolytic signal sequence is prepared downstream of the reporter gene. A peptide linker encoded by the IL-1β partial sequence comprises a sequence recognizable by caspase (i.e., a caspase recognition sequence). Moreover, the peptide linker encoded by the IL-1β partial sequence is a region on which caspase (caspase-1) acts when activated in a protein complex called inflammasome, and this peptide linker is cleaved by the action of caspase.

FIG. **3** shows a schematic view of an inflammation reporter system in cells carrying such a gene construct. FIG.

3 illustrates an embodiment where IL-1β is given as an inflammatory cytokine and luciferase (Luc) is given as a reporter molecule. Of course, inflammatory cytokines and reporter molecules are not limited only to IL-1β and Luc shown in FIG. 3.

In FIG. 3, in the absence of inflammatory stimulation, the IL-1β gene promoter does not function and hence will not activate the expression of the reporter gene. Even if expression leakage occurs, inflammasomes also do not function in the absence of inflammatory stimulation, and the expressed fusion protein composed of a reporter molecule, a caspase recognition sequence and a proteolytic signal sequence will be preferentially degraded through the ubiquitin-proteasome system by the action of the proteolytic signal sequence.

On the other hand, in the presence of inflammatory stimulation, the promoter becomes functional by the action of transcription factor NF-κB to thereby activate the expression of the reporter gene, and the produced reporter molecule is cleaved from the proteolytic signal sequence upon inflammasome-induced activation of caspase, whereby the reporter molecule is stabilized by itself and luminescence signals (reporter signals) from the reporter protein can be detected at high levels. This detection result is visualized and can be confirmed from an image displayed on a monitor.

In one embodiment of the present invention, a gene vector configured to express a fusion protein composed of a reporter molecule, a caspase recognition sequence (which constitutes a portion of the amino acid sequence of IL-1β) and a proteolytic signal sequence under the control of a promoter for the IL-1β gene was transiently introduced into mouse-derived macrophage-like cell line RAW264. When LPS (lipopolysaccharide), which is a component constituting the *E. coli* cell membrane, is added to a culture of this cell line, a significant increase in reporter activity can be observed (FIG. 4).

In another embodiment, this gene vector was injected into a fertilized egg of the C57BL/6 strain at the pronucleus stage to thereby prepare a transgenic mouse. This transgenic mouse is exposed to inflammatory stimulation by being administered intraperitoneally with LPS, and changes in luminescence signals from luciferase are detected with a bioimaging analyzer immediately after administration and at 4 hours and 24 hours after administration. In all tissues of the body, luminescence dependent on inflammatory reaction can be observed (the Example section, FIG. 5).

Examples of a reporter protein (reporter molecule) available for use include luciferase, GFP (green fluorescent protein), DsRed (red fluorescent protein), LacZ (β-galactosidase) and so on. In addition, the gene vector may be in the form of plasmid DNA, viral vector or the like. However, the present invention is not limited to these examples.

Genes encoding these reporter proteins are known and are available from domestic and foreign bioreagent manufacturers, etc.

The term "proteolytic signal sequence" is intended to mean a sequence that is preferentially polyubiquitinated by the action of E3 ligase and thus becomes easily digested in proteasomes. Examples of a proteolytic signal sequence available for use in the present invention include a CL1 sequence, a PEST sequence and so on. Genes encoding these proteolytic signal sequences are known and are available from domestic and foreign bioreagent manufacturers, etc.

The transformant of the present invention may be obtained by introduction of the gene vector into a host.

The host into which the gene vector is introduced is not limited in any way and may be a unicellular organism, as exemplified by prokaryotic organisms (e.g., *E. coli*, lactic acid bacteria) and eukaryotic cells (e.g., yeast). For this purpose, it is also possible to use established cultured cell lines such as human-derived cell lines (e.g., Hela, HEK293) and mouse-derived cell lines (e.g., NIH3T3), or other animal cells. Techniques to ligate the above gene immediately downstream of a promoter are well known (Molecular Cloning: A Laboratory Manual (4th Edition), Cold Spring Harbor Laboratory Press (2012)). Introduction of the gene vector into the host may be accomplished by widely known techniques such as electroporation techniques, lipofection techniques with commercially available lipofection reagents, viral vector-mediated techniques and so on (see, e.g., Molecular Cloning mentioned above).

Transgenic non-human animals transfected with this reporter gene vector may be prepared from mice, rats, dogs, monkeys, goats or other animals, but are not limited to these non-human animals. Transgenic non-human animals may be prepared by injecting the gene vector DNA into fertilized eggs of these respective animals using a microinjector. Alternatively, embryonic stem cells (ES cells) or induced pluripotent stem cells (iPS cells) may be established by homologous recombination and then used to prepare transgenic animals. Microinjection and other techniques are all known techniques which can be easily carried out by those skilled in the art (see, e.g., Molecular Cloning mentioned above).

Transgenic non-human animals used in the present invention are not limited to whole animals, and it is also possible to use biomaterials derived from these transgenic non-human animals, including cells, organs, tissues, embryos, etc.

2. Screening Method

In the present invention, test substances (candidate substances) serving as candidates for anti-inflammatory substances are not limited in any way, and examples include peptides, proteins, DNAs, non-peptidic compounds, synthetic compounds, fermentation products, cell extracts, plant extracts and so on, which may be either novel compounds or known compounds. These test substances may form salts. Salts of test substances include those formed with physiologically acceptable acids (e.g., inorganic acids) or bases (e.g., organic acids), with physiologically acceptable acid addition salts being preferred. Test substances may be tested alone independently of each other or may be tested as a mixture (including a library). Examples of a library containing a plurality of test substances include synthetic compound libraries (e.g., combinatorial libraries), peptide libraries (e.g., combinatorial libraries), etc.

The present invention includes an embodiment where a transgenic non-human animal is administered with an inflammatory substance (exposed to inflammatory stimulation) to induce an inflammatory reaction and this animal is then contacted with a test substance to examine an inhibitory effect on the inflammatory reaction, and an embodiment where a transgenic non-human animal is contacted with a test substance and then administered with an inflammatory substance to induce an inflammatory reaction, followed by examination of an inhibitory effect on the inflammatory reaction in this animal. In either of these embodiments, a test substance found to have an inhibitory effect on the induced inflammatory reaction can be selected as a therapeutic or prophylactic agent for inflammatory diseases (e.g., infections, rheumatism, allergies), i.e., as an anti-inflammatory agent.

A transgenic non-human animal (test animal) to be administered with a test substance and a control animal are not limited in any way, although non-human animals of the same

species are commonly used for this purpose. More preferably, animals of the same sex and of the same age are used as test and control animals.

In the case of using a transformant, the present invention includes an embodiment where the transformant is contacted with a test substance and this transformant is then contacted with an inflammatory substance to examine an inhibitory effect on the induced inflammatory reaction, and an embodiment where the transformant is contacted with an inflammatory substance to induce an inflammatory reaction and this transformant is then contacted with a test substance to examine an inhibitory effect on the inflammatory reaction.

For determination of whether or not an inflammatory reaction is inhibited, it is examined whether or not a reporter protein is detected as a luminescence signal upon inflammatory stimulation, and the resulting detection results are used to select an anti-inflammatory substance.

The term "contact" is intended to include, e.g., an embodiment where a test substance is administered to a non-human animal, an embodiment where a test substance is added to a transformant or a biomaterial, and an embodiment where cells are cultured in the presence of a test substance. To contact a test substance with transgenic non-human animals per se, the test substance may be inoculated into these animals through injection or other means. The embodiment where a test substance is added to a transformant or a biomaterial may be accomplished, e.g., by addition of the test substance to a cultured product of cells or by addition of the test substance to a tissue, an organ or the like. The term "cultured product" is intended to mean a cell, a cell culture or a cell extract. The expression "cultured in the presence of a test substance" is intended to mean that cells are cultured under conditions where the cells are contacted with a test substance, and contact of the test substance with the above cells or the like may be accomplished, e.g., by addition of the test substance to a cell culture medium or any type of buffer (e.g., HEPES buffer, phosphate buffer, phosphate-buffered physiological saline, Tris-HCl buffer) and incubation of the cells therein for a given period of time.

The concentration of a test substance to be added to a cultured product will vary depending on the type of compound (e.g., solubility, toxicity). For example, it is selected as appropriate within the range of 100 ng/ml to 10 μg/ml. The time required for incubation may be, for example, 4 to 48 hours.

3. Kit

The present invention provides a kit for detection of an inflammatory reaction or for screening of anti-inflammatory substances, which comprises a transformant or transgenic non-human animal transfected with a vector comprising a promoter for a gene encoding an inflammatory cytokine, a gene encoding a reporter protein, a gene encoding the inflammatory cytokine, and a gene encoding a proteolytic signal sequence.

In the case of using the transformant or transgenic non-human animal of the present invention as a detection reagent for inflammatory diseases or inflammatory reactions, the kit may comprise the above transformant or transgenic non-human animal together with other reagents, e.g., distilled water, buffering reagents, inflammation-inducing substances, instructions for use, etc.

EXAMPLES

The present invention will be further described in more detail by way of the following examples, which are not intended to limit the scope of the present invention.

Construction of Gene Vector

A region of approximately 5 kbp upstream of the mouse-derived IL-1β gene was cloned from the genomic DNA extracted from mouse-derived cells. An HSV-derived TK gene promoter was fused immediately downstream of the cloned region, *Photinus pyralis*-derived modified luciferase (GL4, approximately 1.7 kbp) was ligated downstream of this fused promoter, a nucleotide sequence encoding a mouse-derived IL-1β partial sequence (17-216 aa) was further ligated downstream thereof, and a CL1 (derived from *Saccharomyces cerevisiae*)-PEST (derived from mouse) sequence and an SV40-derived polyA sequence were further ligated downstream thereof to thereby construct a vector (FIG. 7) (SEQ ID NO: 1).

Cloning of the IL-1β gene was accomplished as follows.

About a Region of Approximately 5 Kbp Upstream of the Mouse-Derived IL-1β Gene

The entire region was divided into 4 portions, and these portions were each cloned by PCR techniques. For each portion, the PCR kit used was Prime Star (Takara), the template DNA used was mouse ES cell-derived genomic DNA, and the reaction conditions were set to 35 cycles of 98° C. for 10 seconds, 55° C. for 5 seconds and 72° C. for 2 minutes. Different primers were used for each portion as shown below.

For the first portion

5-primer: (SEQ ID NO: 2)
aaaactagttcgtcttttgagaaagtcagggcag

3-primer: (SEQ ID NO: 3)
gaataggcatcgataaacaagattc

For the second portion

5-primer: (SEQ ID NO: 4)
gaatcttgtttatcgatgcctattc

3-primer: (SEQ ID NO: 5)
aaactcgaggcacatgcatgaagacgaatggcc

For the third portion

5-primer: (SEQ ID NO: 6)
aaactcgagatgcatgtgccttcctccaaatc

3-primer: (SEQ ID NO: 7)
gtaggagctagcccgggtgagtag

For the fourth portion

5-primer: (SEQ ID NO: 8)
aaaactagttcgtcttttgagaaagtcagggcaggaac

3-primer: (SEQ ID NO: 9)
aaaactagtcacaaggaagcttggctggagaggatc

It should be noted that a ClaI site, an EcoT22I site and a SmaI site were used for ligation of each portion.

About a Partial (17-216 Aa) Region of the Mouse-Derived IL-1β Gene

The entire region was cloned at once by PCR techniques. The PCR kit used was Prime Star (Takara), the template DNA used was a mouse placenta-derived reverse transcript, and the reaction conditions were set to 35 cycles of 98° C. for 10 seconds, 55° C. for 5 seconds and 72° C. for 1 minute.

The primers used were 5-primer: aaaggtaccgatgagaatgac-ctgttctttg (SEQ ID NO: 10) and 3-primer: aaactcgagaaac-cgttntccatcttcttc (SEQ ID NO: 11).

Transient Introduction into Cultured Cells

The gene vector constructed as above was transiently introduced into mouse-derived macrophage-like cell line RAW264.

For transfection, Effectene (Qiagen) was used, and the cells at 24 hours after transfection were collected and provided for experiments. To a culture of transiently expressing cells, LPS (Sigma #L2654) was added at a concentration of 2 μg/mL, and the amount of luciferase luminescence at 48 hours after addition was determined by being measured with a luminometer. For use as a control, an LPS-free group was provided. In addition, as a conventional reporter system detecting only IL-1β gene expression, a vector was prepared to have GL4 ligated downstream of a promoter for the IL-1β gene, and cells transfected with this vector were provided for the same test.

As a result, the reporter signals shown in FIG. **6** were obtained. In the cells transfected either with the vector of the present invention or with the vector of conventional type, a significant increase in reporter signals was observed upon LPS stimulation. However, the vector of the present invention showed a 2-fold or more improvement in sensitivity when compared to the vector of conventional type.

Preparation of Transgenic Mouse

The excised and purified gene vector was injected into 200 fertilized eggs at the pronucleus stage which had been taken from mice of the C57BL/6 strain, thereby obtaining 71 pups. The genomic DNA extracted from the body tissue of each pup was used for genotype analysis to thereby obtain 18 founder mice having the gene vector inserted into their genome. Four founder mice were each crossed with a wild-type mouse of the C57BL/6 strain to produce F1 generation mice, which were then examined for reporter molecule reaction when intraperitoneally administered with LPS (Sigma #L2654).

As a result, the S/N ratio was highest in the mice of strain No. M1, and this strain was established as an inflammation reporter mouse (FIG. **8**).

Visualization of Inflammatory Reactions Using Transgenic Mouse

The inflammation reporter mouse thus established was intraperitoneally administered with LPS (Sigma #L2654) at a concentration of 3 mg/kg wt. At 0, 4 and 24 hours after administration, luciferase luminescence was observed with a bioimaging analyzer (IVIS). As a result, luminescence signals from luciferase were able to be captured from all tissues of the body (FIG. **5**).

Sequence Listing Free Text

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The invention claimed is:

1. A vector comprising an inflammatory cytokine promoter operably linked to a nucleotide sequence comprising a gene encoding a luciferase reporter protein, a gene encoding an inflammatory cytokine, wherein the inflammatory cytokine is a mouse-derived IL-1β partial sequence consisting of amino acids 17-216, and a gene encoding a proteolytic signal sequence,

wherein the luciferase reporter protein, the inflammatory cytokine and the proteolytic signal sequence are configured as a fusion protein, wherein the inflammatory cytokine is N-terminal to the proteolytic signal sequence and C-terminal to the luciferase reporter protein, and

wherein, in the presence of inflammatory stimulation, the inflammatory cytokine promoter becomes functional to thereby activate the expression of the nucleotide sequence and the inflammatory cytokine is cleavable by caspase-1 to separate the luciferase reporter protein from the proteolytic signal sequence while retaining luciferase activity.

2. A non-human transformant comprising the vector according to claim **1**.

3. A transgenic non-human animal comprising in its genome the vector according to claim **1**.

4. The transgenic non-human animal according to claim **3**, wherein the non-human animal is a mouse.

5. The non-human transformant according to claim **2**, wherein the reporter protein is detected as a luminescence signal upon inflammatory stimulation.

6. A method for detection of an inflammatory reaction, which comprises using the transformant according to claim **2** or the transgenic non-human animal according to claim **3**

to detect an inflammatory reaction induced upon inflammatory stimulation in the transformant or in the transgenic non-human animal.

7. The method according to claim **6**, wherein the gene encoding the inflammatory cytokine is expressed by transcription factor NF-κB induced during inflammatory reaction.

8. The method according to claim **6**, wherein the reporter protein is detected as a luminescence signal upon inflammatory stimulation.

9. A method for screening of anti-inflammatory substances, which comprises bringing the transformant according to claim **2** or the transgenic non-human animal according to claim **3** into contact with candidate substances under inflammatory stimulation to select an anti-inflammatory substance on the basis of the presence or absence of an inflammatory reaction serving as an indicator.

10. A kit for detection of an inflammatory reaction or for screening of anti-inflammatory substances, which comprises the non-human transformant according to claim **2**.

11. The vector of claim **1**, wherein the promoter is an interleukin 1β inflammatory cytokine promoter, the reporter protein is *photinus pyralis* luciferase, and the proteolytic signal sequence comprises a CL1-PEST proteolytic signal sequence.

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