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(54) Title: USE OF INTERFERON EPSILON IN METHODS OF DIAGNOSIS AND TREATMENT

(57) Abstract: The present invention relates generally to a method of diagnosing, predicting and/or monitoring the predisposition of a female mammal to reproductive tract infection by a pathogen. More particularly, the present invention relates to a method of diagnosing, predicting and/or monitoring the predisposition of a female mammal to reproductive tract infection by a pathogen by analysing IFN ϵ levels either in said mammal or in a biological sample derived from said mammal. The present invention further provides a method of predicting, diagnosing and/or monitoring the predisposition of a female mammal to conditions associated with or characterised by infection of the reproductive tract with a pathogen.



USE OF INTERFERON EPSILON IN METHODS OF DIAGNOSIS AND TREATMENT**FIELD OF THE INVENTION**

- 5 The present invention relates generally to a method of diagnosing, predicting and/or monitoring the predisposition of a female mammal to reproductive tract infection by a pathogen. More particularly, the present invention relates to a method of diagnosing, predicting and/or monitoring the predisposition of a female mammal to reproductive tract infection by a pathogen by analysing IFN ϵ levels either in said mammal or in a biological
- 10 sample derived from said mammal. The present invention further provides a method of predicting, diagnosing and/or monitoring the predisposition of a female mammal to conditions associated with or characterised by infection of the reproductive tract with a pathogen.
- 15 In a related aspect, the present invention provides a method of eliciting or otherwise inducing an immune response to a pathogen, in particular an innate immune response of the mucosal tissue. More particularly, the present invention relates to a method of eliciting or otherwise inducing an immune response to a pathogen via upregulation of IFN ϵ levels in the mucosal tissue of said mammal. The present invention is useful, *inter alia*, in the
- 20 prophylactic and/or therapeutic treatment of pathogen infections such as, for example, herpes simplex virus, Chlamydia and HIV.

In a further related aspect, there is provided a method for either preventing implantation of a fertilised ovum or inducing the abortion of an implanted embryo by increasing levels of

25 IFN ϵ in the reproductive tract of a female mammal.

BACKGROUND OF THE INVENTION

- The reference in this specification to any prior publication (or information derived from it),
- 30 or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived

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from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Bibliographic details of the publications referred to by author in this specification are
5 collected alphabetically at the end of the description.

Mucosal surfaces are in direct contact with an environment rich in microorganisms. Nevertheless, there is a low incidence of infection and mucosal host defence mechanisms create a hostile environment for potential pathogens. The innate and adaptive immune
10 systems determine host protection throughout mucosal sites, including the female reproductive tract, the respiratory, gastrointestinal and urinary tracts.

The innate immune response represents pre-existing, inherent, first line and rapidly inducible defence to pathogens and responses to homeostatic cues (Mangan *et al. Eur J*
15 *Immunol*, 2007, 37(5):1302-12; Smith *et al. J Immunol*, 2007, 178(7):4557-66). This is mediated through resident cells such as macrophages, NK and epithelial cells. Adaptive immune responses encompass the recognition, and response to antigens with elicited responses being gradual and specific, mediated through antibody secreting B lymphocytes and T helper and effector lymphocytes. The adaptive response is sculpted by the innate
20 system. In the reproductive tract, both arms of the immune system must balance the presence of an allogenic foetus, essentially containing “foreign” proteins, with the control of harmful pathogens e.g. viruses and bacteria. It must also maintain homeostasis against a background of cyclical hormonal milieu and structural changes that occur in the mucosa.

25 The innate and adaptive immune cells of the female reproductive tract produce cytokines and chemokines, thereby influencing various reproductive processes including sperm migration, fertilization, implantation, endometrial remodelling and immune response to infectious challenge (Salamonsen *et al. Semin Reprod Med*, 2007, 25(6):437-44). The cellular populations involved in this process include:

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- Macrophages – phagocytic cells which produce inflammatory cytokines

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- Natural Killer cells – specialised recognition and “killing” requirements on their targets
- Neutrophils –which secrete reactive oxygen radicals and proteases
- Dendritic cells (DC) –which produce cytokines and take up antigens for
5 “presentation” to T cells
- Epithelial cells – produce cytokines and can function as antigen presenting cells to activate T cells
- B cells – which secrete immunoglobulins (Ig) including secretory IgA
- T cells – which are potent producers of specific cytokines and can have specialised
10 ability to “kill” target cells

In its simplest form, the innate response includes physicochemical barriers such as mucous secretions, pH and redox state. In its most sophisticated form it is represented by the innate immune response which senses pathogens within minutes and starts a series of reactions,
15 culminating in the production of products like antimicrobial defensins, NOS enzymes, chemokines that recruit and activate inflammatory cells and cytokines that modulate cell behaviour. The families of innate receptors that orchestrate the innate immune responses are called pathogen recognition receptors (PRRs). These include Toll-like receptors (TLR)
1-13 which sense pathogen cell surface molecules or intracellular nucleic acids; helicases,
20 RIG-I and MDA5 which sense nucleic acids; nuclear oligomerization domain (NOD)–like receptors (NLRs) and newly discovered DNA-sensing molecules. These receptors activate, by recruitment of specific adaptors and activation of enzymes, pathways of two major transcription factors: NF κ B - which induces expression of proinflammatory cytokines such as IL6, TNF α , IL1 and certain cytokines; and Interferon Regulatory Factors (1-9) which
25 induce the expression of type I IFNs.

Interferons (IFN) were discovered over 50 years ago by virtue of their definitive antiviral activity, then subsequently shown to have many biological actions. IFNs are classified into three distinct types based on gene sequence similarity and locus, action through a type-
30 specific receptor and cell or inducer of production. Nevertheless, the IFN types share some intracellular signal transduction mechanisms such as the JAK/STAT pathways and hence

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induce some of the same response genes (IRGs) and consequent biological actions. Type I IFNs consist of multiple subtypes including 14 IFN- α 's, IFN- β , IFN- ω , IFN- κ , and IFN- τ as well as a relatively new subtype designated IFN- ϵ . This locus contains all 14 IFN- α and IFN- β genes and many pseudogenes representing a rapidly evolving locus comprised of
5 related genes with a diversity of promoters that ensure induction of some of this family of critical host defence cytokines in response to a diversity of stimuli. IFNs- α and - β are typically produced by haemopoietic and other cell types in response to virus or bacteria. Accordingly they play a crucial role in protection from viral infection as exemplified by the sensitivity of mice deficient in the type I IFN receptor, *Ifnar1*. The role of type I IFNs
10 in bacterial infections is less clear because *Ifnar1* deficient mice are more susceptible to some infections, and less susceptible to others. In addition to their anti-infection function, type I IFNs have important and diverse effects on cells: regulating cell growth and proliferation, survival, migration and specialised functions. Not surprisingly, therefore, these IFNs are also important in homeostatic processes such as haemopoiesis, bone
15 metabolism and development.

Among the IFN family, some have evolved special functions. For example, in ruminant species there is a unique type I IFN- τ , which is essential for maternal recognition of pregnancy. IFN- τ is produced by trophoblast cells and induces endometrial prostaglandin
20 synthesis and subsequent blockade of corpus luteum. This establishes the precedent for a role for type I IFNs in reproduction and development; although a mammalian homologue of IFN- τ has not been identified. The female reproductive tract is obviously a critical organ which must be receptive to implantation of the fetus and yet be protected from infection. Indeed, the reproductive tract is susceptible to many viral infections such as HPV, HIV and
25 Herpes and bacterial infections such as Chlamydia. In particular, *Chlamydia trachomatis* and Herpes Simplex Virus -2 (HSV-2) are the most prevalent sexually transmitted infections (STIs) worldwide. Despite the extensive studies of immune response to STIs of the female reproductive tract (FRT), including the role of cytokines like the known IFNs, there has been only limited progress in management or prevention of these diseases.

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In work leading up to the present invention it has been determined that IFN ϵ is

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constitutively expressed in the reproductive tract of female mammals and that deficiency in IFN ϵ levels leads to susceptibility to pathogenic infection of the reproductive tract. Still further, it has been determined that IFN ϵ is not regulated by stimulation of pathogen recognition receptors, including toll-like receptors, nor by co-expression with IRF

5 transcription factors which are known to regulate IFN α/β expression. IFN ϵ nevertheless functions to induce mucosal tissue innate immune responsiveness. Accordingly, these findings have facilitated the development of both a method of screening female mammals for susceptibility to reproductive tract infection and, further, therapeutically or prophylactically eliciting an immune response to a pathogen via the upregulation of IFN ϵ

10 levels in the mucosa, thereby stimulating innate mucosal immunity. Still further, the determination that IFN ϵ levels are regulated via the estrus cycle has enabled the development of means for both preventing embryo implantation or inducing the abortion of an implanted embryo in a female mammal.

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SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”,
5 will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

As used herein, the term “derived from” shall be taken to indicate that a particular integer or group of integers has originated from the species specified, but has not necessarily been
10 obtained directly from the specified source. Further, as used herein the singular forms of “a”, “and” and “the” include plural referents unless the context clearly dictates otherwise.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this
15 invention belongs.

One aspect of the present invention is directed to a method of screening for a predisposition to developing a pathogen infection of the reproductive tract in a female mammal said method comprising measuring the level of expression of IFN ϵ in a biological
20 sample from said mammal wherein a lower level of expression of said IFN ϵ relative to control levels is indicative of a predisposition to developing a pathogen infection of the reproductive tract.

In another aspect there is provided a method of screening for a predisposition to
25 developing a microorganism infection of the reproductive tract in a female mammal said method comprising measuring the level of expression of IFN ϵ in a biological sample from said mammal wherein a lower level of expression of said IFN ϵ relative to control levels is indicative of a predisposition to developing a microorganism infection of the reproductive tract.

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In yet another aspect the present invention provides a method of screening for a

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predisposition to developing a pathogen infection of the reproductive tract in a female mammal said method comprising measuring the level of IFN ϵ protein in a biological sample from said mammal wherein a lower level of IFN ϵ protein relative to control levels is indicative of a predisposition to developing a pathogen infection of the reproductive tract.

In still another aspect there is provided a method of screening for a predisposition to developing a pathogen infection of the reproductive tract in a female mammal said method comprising measuring the level of expression of IFN ϵ in a blood sample or vaginal swab from said mammal wherein a lower level of expression of said IFN ϵ relative to control levels is indicative of a predisposition to developing a pathogen infection of the reproductive tract.

Another aspect of the present invention relates to a method for monitoring a females mammal's predisposition to developing a pathogen infection of the reproductive tract, said method comprising measuring the level of expression of IFN ϵ in a biological sample from said mammal wherein a lower level of expression of said IFN ϵ relative to control levels is indicative of a predisposition to developing a pathogen infection of the reproductive tract.

A further aspect of the present invention is directed to a method of eliciting or inducing, in a female mammal exposed to a pathogen, a mucosal immune response, said method comprising the administration of an effective amount of a composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .

In a related aspect there is provided a method for the prophylactic and/or therapeutic treatment of a condition characterised by a pathogen infection in a female mammal, said method comprising the administration of an effective amount of a composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .

In a further aspect there is provided the use of a composition, which composition comprises an agent which upregulates the level of IFN ϵ , in the manufacture of a

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medicament for the prophylactic or therapeutic treatment of a condition in a female mammal characterised by a pathogen infection.

5 In another further aspect there is provided a method of eliciting or inducing, in a female mammal exposed to a pathogen, an innate immune response, said method comprising the mucosal administration of an effective amount of composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .

10 In a related aspect there is provided a method for the prophylactic and/or therapeutic treatment of a condition characterised by a pathogen infection in a female mammal, said method comprising the mucosal administration of an effective amount of a composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .

15 In a further aspect there is provided the use of a composition, which composition comprises an effective amount of an agent which upregulates the level of IFN ϵ , in the manufacture of a medicament for the prophylactic or therapeutic treatment of a condition characterised by a pathogen infection, wherein said medicament is mucosally administered.

20 In yet another further aspect there is provided a method of eliciting or inducing, in a female mammal exposed to a microorganism, an innate mucosal immune response, said method comprising the mucosal administration of an effective amount of composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .

25 In a related aspect there is provided a method for the prophylactic and/or therapeutic treatment of a condition characterised by a microorganism infection in a mammal, said method comprising the mucosal administration of an effective amount of composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .

30 In a further aspect there is provided the use of a composition, which composition comprises an effective amount of an agent which upregulates the level of IFN ϵ , in the

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manufacture of a medicament for the prophylactic or therapeutic treatment of a condition characterised by a microorganism infection, wherein said medicament is mucosally administered.

- 5 In still a further aspect there is provided a method of eliciting or inducing an immune response directed to a pathogen, said method comprising the administration of an effective amount of a composition wherein said composition comprises an agent which upregulates the level of IFN ϵ together with one or more pathogen derived antigens.
- 10 In still yet a further aspect there is provided the use of a composition in the manufacture of a vaccine, which composition comprises an effective amount of an agent which upregulates the level of IFN ϵ together with one or more pathogen derived antigens.

- Still another aspect of the present invention is directed to a composition suitable for
- 15 inducing a mucosal immune response to a pathogen, said composition comprising an agent which upregulates the level of IFN ϵ together with one or more pathogen derived antigens.

- In yet another aspect there is provided a vaccine suitable for inducing a mucosal immune response to a pathogen, said vaccine comprising an agent which upregulates the level of
- 20 IFN ϵ together with one or more pathogen derived antigens.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphical representation demonstrating that *Ifn-ε* is not induced by Simliki Forest virus stimulation and LPS infection. (A) Raw264.5 cells were treated with Simliki Forest virus at the different time point (0hr, 3hr, 6hr, 9hr, 15hr and 24hr). Total RNA was extracted and *Ifn-ε* and 2'5' oas expressions were measured by Taqman qRT-PCR.

Figure 2 is a graphical representation demonstrating that *Ifn-β* and *Ifnα* are not expressed constitutively in the mouse organs. Different mouse organs (as indicated) were harvested. Total RNA was extracted and *Ifn-β* expression was analysed by Taqman qRT-PCR while *Ifnα* expression was analysed by SyberGreen qRT-PCR.

Figure 3 is a graphical representation demonstrating that *Ifn-ε* exhibits a distinct expression pattern. (A) Different mouse organs (n=3) (as indicated) were harvested, (B-C) uteri from mice (n=3) was harvested at different estrus stage (B) and time of pregnancy (C). Total RNA was extracted and *Ifn-ε* expression was analyzed by Taqman qRT-PCR. Raw data was normalized to 18S ribosomal RNA to correct for variations in mRNA prior to calculation of fold induction.

Figure 4 is an image relating to the generation of *Ifn-ε*^{-/-} mice. (A) The wild type allele, targeting vector and the targeted allele are depicted. *Ifn-ε* exon is represented by black rectangle. The black triangle and purple rectangle indicate the loxP sites and neo cassette respectively. BamI (B) and SacI (S) restriction sites used for assessment of 5' and 3' homologous recombination are shown. (B) Southern Blot analysis on targeted EScells. (C) PCR screen on wild type and targeted flp'd alleles. (D) Genotyping PCR from tail genomic DNA. (E) uteri from *Ifn-ε*^{-/-} mice were harvested and the expression of *Ifn-ε* was analyzed by Taqman qRT-PCR. (F) The scatter plot showing the number of pups from different mating pairs at different number of litter.

Figure 5 is a representation depicting that *Ifn-ε*^{-/-} mice has normal T cells and B cells development and normal uterine morphology. (A) Spleen and (B) Thymus were taken from

both Ifn- $\epsilon^{-/-}$ and Ifn- $\epsilon^{+/+}$ and different T cell populations, CD4⁺CD8⁻, CD4⁻CD8⁺ and CD4⁺CD8⁺, were analyzed by flow cytometric analysis (n=5). (C) Bone marrow was taken from Ifn- $\epsilon^{-/-}$ and Ifn- $\epsilon^{+/+}$ and different B cells populations, IgD⁺IgM⁺ (Mature B cells) IgD⁻IgM⁺ (Immature B cells), were analyzed by flow cytometry (n=5).

5

Figure 6 is a graphical representation of the expression of certain IRG subsets are reduced in the uterus of Ifn- $\epsilon^{-/-}$ mice. RNA was harvested from the Uterine horn at Estrus stage of Ifn- $\epsilon^{+/+}$ and Ifn- $\epsilon^{-/-}$ mice. (A) 2'5' *oas*, (B) *isg15*, (C) *irgm1* and (D) *Irf7* expressions were analyzed by Taqman RT-PCR.

10

Figure 7 is a graphical representation depicting that the IRG expressions show no difference in the kidney of Ifn- $\epsilon^{-/-}$ mice. RNA was harvested from the kidney of Ifn- $\epsilon^{+/+}$ and Ifn- $\epsilon^{-/-}$ mice. (A) 2'5' *oas*, (B) *isg15*, (C) *irgm1* expressions were analyzed by Taqman RT-PCR.

15

Figure 8 depicts that Ifn- $\epsilon^{-/-}$ mice are more susceptible to *Chlamydia Muridarum* vaginal infection. (A) Clinical Scores including mucus production, swelling and redness of the vagina and coat were recorded daily for 30 days. (B) Bacterial recovery from the vaginal swab of Ifn- $\epsilon^{+/+}$ and Ifn- $\epsilon^{-/-}$ mice at different time points (day 4, 7, 14, 21 and 29). (C) RNA was harvested from the uterine horn of Ifn- $\epsilon^{+/+}$ and Ifn- $\epsilon^{-/-}$ mice at day 30, bacterial 16S gene was examined by Taqman RT-PCR. (D) Bacterial recovery from the vaginal lavage at day 1 and 3 p.i. (E) RNA was harvested from the uterine horn of Ifn- $\epsilon^{+/+}$ mice at day 1, 3 and 30 of post infection, Ifn- ϵ expression was analyzed by Taqman RT-PCR.

20

Figure 9 depicts that Ifn- $\epsilon^{-/-}$ mice are more susceptible to HSV-2 vaginal infection. (A) Weight changes of Ifn- $\epsilon^{+/+}$ and Ifn- $\epsilon^{-/-}$ mice during the course of infection. (B) Clinical Score of Ifn- $\epsilon^{+/+}$ and Ifn- $\epsilon^{-/-}$ mice during the course of infection. (C) Overt genital lesion in HSV-2 infected Ifn- $\epsilon^{-/-}$ mice at day 7p.i. compare with Ifn- $\epsilon^{+/+}$ mice. HSV-2 titres from vaginal tissue obtained from infected Ifn- $\epsilon^{+/+}$ and Ifn- $\epsilon^{-/-}$ mice (n=5) at (D) day 3 p.i. Data represent cumulative means $\pm$ SEM from 2 to 3 separate experiments (n=5). *, P values of <0.05 for comparison of Ifn- $\epsilon^{+/+}$ to Ifn- $\epsilon^{-/-}$ mice, as determined by student t test.

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Figure 10 is a graphical representation depicting that *Ifn-ε* is not induced by TLR ligands stimulation or regulated by IRFs. (A) Raw264.5 cells were treated as indicated in the following concentration: LPS, Pam3Cys, PolyIC, CpGDNA and Loxoribine. Total RNA
 5 was extracted 3 h posttreatment and *Ifn-ε*, *Ifn-β* and *il-6* expressions were measured by Taqman qRT-PCR. The data are presented as mean + SE of three independent experiments, performed in triplicates. Fold induction is shown relative to untreated cells. Raw data was normalized to 18S ribosomal RNA to correct for variations in mRNA prior to calculation of fold induction. (B) Hek293 cells were cotransfected with *Irf3-5D*, *Irf7*
 10 and *Irf5* expression plasmid and luciferase reporter construct containing the *Ifn-β*, *-ε*, *-α* and *p125*. The luciferase activities were measured at 24 hrs posttransfection and are expressed as fold induction relative to the basal level. In each case the promoterless pGL3 vector was used as a negative control. The results were normalized by using Renilla luciferase. The result represents the average of three experiments, performed in triplicates, with variability
 15 (mean ± the SE) shown by error bars.

Figure 11 is a schematic representation of the *Ifn-ε* promoter from different species showing some of the predicted transcription factor binding sites that are conserved across species. Transcription factors binding sites including Ets, PLZF, BRCA1, STAT, GR and
 20 IRF2 were shown by different colours. Most of these transcription factor binding sites are conserved across different species such as *Mus musculus*, *Homo sapiens*, *Rattus norvegicus*, *Bos taurus*, *Canis domesticus* and *Pan troglodytes*.

Figure 12 is a graphical representation of the comparative biological activities of IFNε *in vitro*. ISG15 = interferon stimulated gene 15; IRF7 = interferon regulatory factor 7;
 25 250AS = 2'-5' oligoadenylate synthetase.

Figure 13 is an image which depicts the production and characterisation of recombinant IFNε. A. IFNε has approximately 30% amino acid identity to IFNs α and also to IFNβ.
 30 B. Visualisation of purified recombinant IFNε. Left hand lane is a Coomassie stained gel; right hand lane is Western blot using specific IFNε antibodies.

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Figure 14 is a graphical representation of the *in vivo* immunomodulatory activities of IFN ϵ .

- 5 **Figure 15** is a graphical representation of IFN γ , ISG15, TNF α , IL6, CCL3 and CCL2 expression following intraperitoneal IFN treatment.

Figure 16 is a graphical representation depicting TNF α and CCL2 expression following intravaginal IFN treatment.

10

Figure 17 shows that IFN ϵ is induced by TGF β (mean and range of 2 experiments).

Figure 18 shows that the B cell levels in spleen are reduced in IFN ϵ -/-mice demonstrating that IFN ϵ can regulate numbers.

15

Figure 19 shows that after infection of mice with HSV-2, the levels of T and B cells in spleen are different –indicating a role for IFN ϵ regulation. Also levels in the regional lymph node of T cells, Dendritic cells, NK cells and monocytes are different. This data again indicates a role for IFN ϵ in regulating these populations during a viral infections -
20 definitely relevant to vaccine or anti-infection applications.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated, in part, on the determination that IFN ϵ is constitutively produced in the reproductive tract of the female mammal. Since it functions to induce
5 innate immune responsiveness in the mucosal tissue, without any requirement for stimulation by pathogen recognition receptors, there has been facilitated the development of a method for prophylactically or therapeutically inducing an immune response to a pathogen based on upregulating levels of IFN ϵ in the mucosal tissue thereby stimulating innate mucosal immunity. There has also been facilitated the development of a method of
10 screening female mammals for susceptibility to pathogen infection of the female reproductive tract based on detecting the downregulation of IFN ϵ levels in said mammals.

Accordingly, one aspect of the present invention is directed to a method of screening for a predisposition to developing a pathogen infection of the reproductive tract in a female
15 mammal said method comprising measuring the level of expression of IFN ϵ in a biological sample from said mammal wherein a lower level of expression of said IFN ϵ relative to control levels is indicative of a predisposition to developing a pathogen infection of the reproductive tract.

20 Reference to the “reproductive tract” of a female mammal should be understood as a reference to the organs and tissues which contribute towards the reproductive process. Without limiting the present invention to any one theory or mode of action, the female reproductive tract comprises three main parts: the vagina, the uterus, and the ovaries. The vagina meets the outside at the vulva, which also includes the labia, clitoris and urethra.
25 The vagina is attached to the uterus through the cervix, while the uterus is attached to the ovaries via the fallopian tubes. At certain intervals, typically approximately every 28 days, the ovaries release an ovum, which passes through the fallopian tube into the uterus. The lining of the uterus, called the endometrium, and unfertilized ova are shed each cycle through the process known as menstruation. Reference to “reproductive tract” should
30 therefore be understood as a reference to all or part of any one or more of these organs or tissues.

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Reference to “infection” should be understood as a reference to the detrimental or otherwise unwanted colonisation of the reproductive tract of a female mammal by a pathogen. To this end, the subject infection may occur in any one or more regions, or
5 parts, of the reproductive tract (such as in the context of one or more organs or one or more tissue regions). It may also be an infection which, although being evident in all or part of the reproductive tract, is also found in non-reproductive tract tissues, regions or organs of the mammal. For example, although HIV infection can be introduced via the reproductive tract tissue, it is ultimately an infection which spreads systemically. Still further reference
10 to an infection “of” the reproductive tract should be understood to encompass an infection which is only transiently present in the reproductive tract, such as an infection which enters the body by this route but ultimately localises elsewhere.

Reference to “pathogen” should be understood as a reference to any agent which causes
15 disease or the presence of which is otherwise unwanted in a mammal. For example said pathogen may be a microorganism, such as a bacterium, virus, fungus or parasite. Alternatively, said pathogen may be a non-living agent, such as a synthetically generated or naturally occurring toxin, synthetically generated or naturally occurring environmental antigen, prion or any other proteinaceous or non-proteinaceous molecule, the clearance or
20 neutralisation of which via the induction of an immune response. In one embodiment, said pathogen is a microorganism.

According to this embodiment, there is provided a method of screening for a predisposition to developing a microorganism infection of the reproductive tract in a female mammal
25 said method comprising measuring the level of expression of IFN ϵ in a biological sample from said mammal wherein a lower level of expression of said IFN ϵ relative to control levels is indicative of a predisposition to developing a microorganism infection of the reproductive tract.

30 In another embodiment, said microorganism is a microorganism which is capable of sexual transmission.

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In yet another embodiment, said microorganism is herpes simplex virus, human papilloma virus, human immunodeficiency virus or Chlamydia trachomatis.

5 Reference to "IFN ϵ " should be understood as a reference to all forms of IFN ϵ and to fragments, mutants or variants thereof. Without limiting the present invention to any one theory or mode of action, IFN ϵ belongs to the type I interferon family. Mature IFN ϵ comprises 187 amino acids. Human IFN ϵ genes are clustered on chromosome 9p21.2, close to the location of other type I interferon loci. IFN ϵ has amino acid homology with
10 other type I interferons, of which IFN β is the closest paralog and they share 38% identical residues. Compared with other type I α interferons, there is an extended C end in IFN ϵ . A disulfide bond (Cys32-Cys140) has been predicted to form between the top of AB loop and E helix which is the typical structure of type I α interferons. However, similarly to IFN β , IFN ϵ also lacks the disulfide bond between A and C helices. The two glycosylation sites
15 of IFN ϵ are on asparagine 74 and 83, which locate on the connection loop between B and C helices. A glycosylation site of IFN β also locates in this region.

Reference to IFN ϵ should be understood to include reference to all forms of this molecule including precursor, proprotein, or intermediate forms. It also includes reference to any
20 isoforms which may arise from alternative splicing of IFN ϵ mRNA or polymorphic forms of IFN ϵ . Reference to IFN ϵ extends to any IFN ϵ protein, whether existing as a dimer, multimer or fusion protein.

The term "mammal" as used herein includes humans, primates, livestock animals (eg.
25 horses, cattle, sheep, pigs, donkeys), laboratory test animals (eg. mice, rats, guinea pigs), companion animals (eg. dogs, cats) and captive wild animals (eg. kangaroos, deer, foxes). Preferably the mammal is a human or a laboratory test animal. Even more preferably, the mammal is a human.

30 The present invention is predicated on the determination that IFN ϵ is constitutively

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expressed in the female reproductive tract and is involved in the induction of innate immune responsiveness. The “classical” type I IFNs (IFN α and IFN β) are not constitutively expressed but are rapidly inducible upon infection. Strong IFN responses are induced by activation of most pathogen pattern recognition receptors including toll-like
5 receptors (TLRs), retinoic acid-inducible (RIG) gene-like helicases (RLHs) and nucleotide and oligomerization domain (NOD)-like receptors (NLRs). They then bind to type I IFN receptors (IFNARs) and induce the production of an array of signalling molecules and anti-pathogen immune factors. This occurs through the phosphorylation and activation of
10 Transcription (STAT) family, which translocate to the nucleus and modulate the expression of IFN regulated genes (IRGs).

Without limiting the present invention to any one theory or mode of action, unlike IFN α and IFN β , however, IFN ϵ is regulated independently of pathogens and toll-like receptors.
15 Specifically, it has been determined that IFN ϵ is hormonally regulated. In the absence of IFN ϵ expression, the innate immune response which is localised to the female reproductive tract is downregulated and the mammal becomes vulnerable to pathogen infection. The method of the present invention can therefore identify female mammals who are predisposed to contracting pathogen infections of the female reproductive tract due to
20 inadequate functioning of the innate immune response. This is extremely valuable since it enables decisions to be made regarding the prophylactic or therapeutic treatment of a mammal who is determined not to have an adequately functioning innate immunity in the reproductive tract.

25 In terms of the method of the present invention, screening for the “level of expression” of IFN ϵ may be achieved in a variety of ways including screening for IFN ϵ protein, mRNA, primary RNA or cDNA generated therefrom. Changes to the levels of any of these products is indicative of changes to the expression of IFN ϵ protein levels. In one embodiment, said level of expression is the level of expression of the IFN ϵ protein.

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According to this embodiment, the present invention provides a method of screening for a

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predisposition to developing a pathogen infection of the reproductive tract in a female mammal said method comprising measuring the level of IFN ϵ protein in a biological sample from said mammal wherein a lower level of IFN ϵ protein relative to control levels is indicative of a predisposition to developing a pathogen infection of the reproductive tract.

In another embodiment, said pathogen is a microorganism.

In yet another embodiment, said microorganism is one which is capable of sexual transmission, such as human immunodeficiency virus, human papilloma virus, herpes simplex virus or chlamydia.

The method of the present invention is predicated on the comparison of the level of IFN ϵ in a biological sample with a control level of IFN ϵ . The "control level" is either:

- (i) the level of IFN ϵ expressed in the reproductive tract of a healthy mammal which is at the same stage of the estrus cycle as the mammal who is the subject of screening; or
- (ii) a specific level of IFN ϵ selected by the skilled person. This level may be, for example, the maximum level of IFN ϵ which is known to be expressed during the estrus cycle or it may be a lesser level below which it is believed innate immunity becomes compromised. It is within the skill of the person of skill in the art to determine relevant control level of IFN ϵ .

The control level may be a standard result which reflects individual or collective results obtained from individuals other than the mammal in issue. This form of analysis is in fact a preferred method of analysis since it enables the design of kits which require the collection and analysis of a single biological sample, being a test sample of interest. The standard results which provide the control level may be calculated by any suitable means which would be well known to the person of skill in the art. For example, a population of

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normal tissues can be assessed in terms of the level of the IFN ϵ , thereby providing a standard value or range of values against which all future test samples are analysed. It should also be understood that the control level may be determined from the subjects of a specific cohort and for use with respect to test samples derived from that cohort.

- 5 Accordingly, there may be determined a number of standard values or ranges which correspond to cohorts which differ in respect of characteristics such as age, gender, ethnicity or health status. Said "control level" may be a discrete level or a range of levels.

The screening method of the present invention can be performed on any suitable biological
10 sample. To this end, reference to a "biological sample" should be understood as a reference to any sample of biological material derived from a mammal such as, but not limited to, cellular material, biological fluids (eg. blood, vaginal fluids), tissue biopsy specimens, swab specimens (such as a vaginal swab), surgical specimens or fluid which has been introduced into the body of an animal and subsequently removed (such as, for
15 example, the solution retrieved from a vaginal douche). The biological sample which is tested according to the method of the present invention may be tested directly or may require some form of treatment prior to testing. For example, a tissue sample may require homogenisation prior to testing or it may require sectioning for *in situ* testing of the qualitative expression levels of IFN ϵ genes. Alternatively, a cell sample may require
20 permeabilisation prior to testing. Further, to the extent that the biological sample is not in liquid form, (if such form is required for testing) it may require the addition of a reagent, such as a buffer, to mobilise the sample.

The biological sample may be directly tested or else all or some of the nucleic acid or
25 protein material present in the biological sample may be isolated prior to testing. To this end, and as hereinbefore described, it would be appreciated that when screening for changes to the level of expression of IFN ϵ , one may screen for a translated expression product or the RNA transcripts themselves or cDNA which has been transcribed therefrom. In yet another example, the sample may be partially purified or otherwise enriched prior to
30 analysis. For example, to the extent that a biological sample comprises a very diverse composition, it may be desirable to enrich for a component of particular interest, such as

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the soluble protein fraction. It is within the scope of the present invention for the target cell population or molecules derived therefrom to be pretreated prior to testing, for example, inactivation of live virus or being run on a gel. It should also be understood that the biological sample may be freshly harvested or it may have been stored (for example by
5 freezing) prior to testing or otherwise treated prior to testing (such as by undergoing culturing).

The choice of what type of sample is most suitable for testing in accordance with the method disclosed herein will be dependent on the nature of the situation. Preferably, said
10 sample is a blood sample, vaginal wash or vaginal swab.

There is therefore provided a method of screening for a predisposition to developing a pathogen infection of the reproductive tract in a female mammal said method comprising measuring the level of expression of IFN ϵ in a blood sample or vaginal swab from said
15 mammal wherein a lower level of expression of said IFN ϵ relative to control levels is indicative of a predisposition to developing a pathogen infection of the reproductive tract.

In one embodiment, said pathogen is a microorganism. In another embodiment, said microorganism is capable of sexual transmission, such as human papilloma virus, human
20 immunodeficiency virus, herpes simplex virus or chlamydia.

In another embodiment, said IFN ϵ is the IFN ϵ protein.

Although the preferred method is to detect a decrease in IFN ϵ levels in order to diagnose a
25 predisposition to developing a pathogen infection of the female reproductive tract, a detection of an increase in the level of IFN ϵ may be desired under certain circumstances. For example, to monitor for improvement in IFN ϵ levels, and by implication, innate immunity, during the course of prophylactic or therapeutic treatment of a patient found to be at risk of pathogen infection.

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Accordingly, another aspect of the present invention relates to a method for monitoring a

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females mammal's predisposition to developing a pathogen infection of the reproductive tract, said method comprising measuring the level of expression of IFN ϵ in a biological sample from said mammal wherein a lower level of expression of said IFN ϵ relative to control levels is indicative of a predisposition to developing a pathogen infection of the reproductive tract.

In one embodiment, said pathogen is a microorganism. In another embodiment, said microorganism is capable of sexual transmission.

10 In yet another embodiment, said IFN ϵ is the IFN ϵ protein.

In still another embodiment, said biological sample is a blood sample or a vaginal swab.

The method of the present invention is therefore useful as a one off test or as an on-going monitor of those individuals thought to be at risk of pathogen infection of the reproductive tract or as a monitor of the effectiveness of therapeutic or prophylactic treatment regimes directed to normalising IFN ϵ levels and thereby improving the innate immune system of the reproductive tract. In these situations, mapping the modulation of IFN ϵ levels in any one or more classes of biological samples is a valuable indicator of the status of an individual or the effectiveness of a therapeutic or prophylactic regime which is currently in use. Accordingly, the method of the present invention should be understood to extend to monitoring for increases or decreases in IFN ϵ expression levels in an individual relative to their normal level or relative to one or more earlier expression levels determined from a biological sample of said individual.

25

Means of testing for IFN ϵ expression in a biological sample can be achieved by any suitable method, which would be well known to the person of skill in the art, such as but not limited to:

30 (i) Measurement of altered IFN ϵ protein levels in biological extracts, for example by immunoassay.

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Testing for proteinaceous IFN ϵ expression product in a biological sample can be performed by any one of a number of suitable methods which are well known to those skilled in the art. Examples of suitable methods include, but are not limited to, antibody based screening of tissue sections, biopsy specimens or bodily fluid samples.

To the extent that antibody based methods of diagnosis are used, the presence of IFN ϵ protein may be determined in a number of ways such as by Western blotting, ELISA or flow cytometry procedures. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent.

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In the typical forward sandwich assay, a first antibody having specificity for the IFN ϵ or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking, covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the antigen. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the antigen.

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

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In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of antigen which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well

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established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

- 5 (ii) Determining altered IFN ϵ protein expression based on any suitable functional test, enzymatic test or immunological test in addition to those detailed in point (i) above.
- (iii) Detection of up-regulation of PCR expression in the cells by Fluorescent *In situ* Hybridization (FISH), or in extracts from tissues by technologies such as
10 Quantitative Reverse Transcriptase Polymerase Chain Reaction (QRT-PCR) or Flow cytometric qualification of competitive RT-PCR products (Wedemeyer *et al.*, *Clinical Chemistry* 48:9 1398-1405, 2002).
- (iv) Assessment of expression profiles of RNA, for example by array technologies
15 (Alon *et al.*, *Proc. Natl. Acad. Sci. USA*: 96, 6745-6750, June 1999).

A "microarray" is a linear or multi-dimensional array of preferably discrete regions, each having a defined area, formed on the surface of a solid support. The density of the discrete regions on a microarray is determined by the total numbers of target
20 polynucleotides to be detected on the surface of a single solid phase support. As used herein, a DNA microarray is an array of oligonucleotide probes placed onto a chip or other surfaces used to detect complementary oligonucleotides from a complex nucleic acid mixture. Since the position of each particular group of probes in the array is known, the identities of the target polynucleotides can be determined
25 based on their binding to a particular position in the microarray.

Recent developments in DNA microarray technology make it possible to conduct a large scale assay of a plurality of target nucleic acid molecules on a single solid phase support. U.S. Pat. No. 5,837,832 (Chee *et al.*) and related patent applications
30 describe immobilizing an array of oligonucleotide probes for hybridization and detection of specific nucleic acid sequences in a sample. Target polynucleotides of

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interest isolated from a tissue of interest are hybridized to the DNA chip and the specific sequences detected based on the target polynucleotides' preference and degree of hybridization at discrete probe locations. One important use of arrays is in the analysis of differential gene expression, where the profile of expression of genes in different cells or tissues, often a tissue of interest and a control tissue, is compared and any differences in gene expression among the respective tissues are identified. Such information is useful for the identification of the types of genes expressed in a particular tissue type and diagnosis of conditions based on the expression profile.

10 In one example, RNA from the sample of interest is subjected to reverse transcription to obtain labelled cDNA. See U.S. Pat. No. 6,410,229 (Lockhart *et al.*) The cDNA is then hybridized to oligonucleotides or cDNAs of known sequence arrayed on a chip or other surface in a known order. In another example, the RNA is isolated from a biological sample and hybridised to a chip on which are anchored cDNA probes. The location of the oligonucleotide to which the labelled cDNA hybridizes provides sequence information on the cDNA, while the amount of labelled hybridized RNA or cDNA provides an estimate of the relative representation of the RNA or cDNA of interest. See Schena, *et al. Science* 270:467-470 (1995). For example, use of a cDNA microarray to analyze gene expression patterns in human cancer is described by DeRisi, *et al. (Nature Genetics* 14:457-460 (1996)).

(v) *In vivo* detection.

25 Molecular Imaging may be used following administration of imaging probes or reagents capable of disclosing altered expression of IFN ϵ in the intestinal tissues. Molecular imaging (Moore *et al.*, *BBA*, 1402:239-249, 1988; Weissleder *et al.*, *Nature Medicine* 6:351-355, 2000) is the *in vivo* imaging of molecular expression that correlates with the macro-features currently visualized using "classical" diagnostic imaging techniques such as X-Ray, computed tomography (CT), MRI, Positron Emission Tomography (PET) or endoscopy.

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A person of ordinary skill in the art could determine, as a matter of routine procedure, the appropriateness of applying a given method to a particular type of biological sample.

- 5 In a related aspect, the present findings have enabled the development of means for prophylactically or therapeutically treating pathogen infections or any other condition which would benefit from upregulation of a mucosal innate immune response. Without limiting the present invention to any one theory or mode of action, the innate immune system is the first line of defence against invading microorganisms. In its simplest form, the innate response includes physicochemical barriers such as mucous secretions, pH and redox state. In its most sophisticated form it is represented by the innate immune response which senses pathogens within minutes and starts a series of reactions, culminating in the production of products like antimicrobial defensins, NOS enzymes, chemokines that recruit and activate inflammatory cells and cytokines that modulate cell behaviour. The families of innate receptors that orchestrate the innate immune responses are called pathogen recognition receptors (PRRs). These include Toll-like receptors (TLR) 1-13 which sense pathogen cell surface molecules or intracellular nucleic acids; helicases, RIG-I and MDA5 which sense nucleic acids; nuclear oligomerization domain (NOD)-like receptors (NLRs) and newly discovered DNA-sensing molecules. These receptors activate, by recruitment of specific adaptors and activation of enzymes, pathways of two major transcription factors: NF κ B - which induces expression of proinflammatory cytokines such as IL6, TNF α , IL1 and certain cytokines; and Interferon Regulatory Factors (1-9) which induce the expression of type I IFNs.
- 25 However, the innate immune system alone is not always able to clear infections. The adaptive or specific immune response is activated if the invading microorganism cannot be eliminated or neutralised by innate immune system complexes. Two major features characterise the adaptive immune response, (i) the immune response is antigen specific with the specificity resulting from the use of clonally distributed antigen receptors, and (ii) the development of memory which allows the rapid response of antigen-specific effector cells upon second encounter of the relevant antigen. To this end, in addition to providing a

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first line of defence, the innate immune system also plays an important role in supporting the functioning of the adaptive immune response. Specifically, the innate immune system acts in this regard by:

- 5 • Recruiting immune cells to sites of infection through the production of chemical factors, such as cytokines.
- Activation of the complement cascade to identify bacteria, activate cells and to promote clearance of dead cells or antibody complexes.
- The identification and removal of foreign substances present in organs, tissues, the
10 blood and lymph via phagocytosis.
- Activation of the adaptive immune system through antigen presentation.

Accordingly, this aspect of the present invention is directed to a method of eliciting or inducing, in a female mammal exposed to a pathogen, a mucosal immune response, said
15 method comprising the administration of an effective amount of a composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .

In a related aspect there is provided a method for the prophylactic and/or therapeutic treatment of a condition characterised by a pathogen infection in a female mammal, said
20 method comprising the administration of an effective amount of a composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .

In a further aspect there is provided the use of a composition, which composition comprises an agent which upregulates the level of IFN ϵ , in the manufacture of a
25 medicament for the prophylactic or therapeutic treatment of a condition in a female mammal characterised by a pathogen infection.

In one embodiment, said immune response is an innate immune response.

30 In another embodiment, said pathogen is a microorganism.

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Reference to “IFN ϵ ” should be understood to have the same meaning as hereinbefore provided.

As discussed hereinbefore, IFN ϵ has been determined to function in the mucosal tissue of
5 the female reproductive tract to facilitate the functioning of the mucosal innate immune response. Accordingly, in one embodiment said IFN ϵ is targeted to the mucosal tissue.

Without limiting the present invention to any one theory or mode of action, the
administration of antigen to a mucosal site will induce the onset of a mucosal immune
10 response. The mucosal immune system in mammals consists of an integrated network of lymphoid tissues and mucosal membrane-associated cells and effector molecules that work together to achieve host protection. Major effector molecules include antibodies, such as the IgA isotype, as well as cytokines, chemokines and their receptors which function in synergy with the innate response which is initially stimulated by the method of the present
15 invention.

According to this embodiment there is provided a method of eliciting or inducing, in a female mammal exposed to a pathogen, an innate immune response, said method comprising the mucosal administration of an effective amount of composition wherein said
20 composition comprises an agent which upregulates the level of IFN ϵ .

In a related aspect there is provided a method for the prophylactic and/or therapeutic treatment of a condition characterised by a pathogen infection in a female mammal, said method comprising the mucosal administration of an effective amount of a composition
25 wherein said composition comprises an agent which upregulates the level of IFN ϵ .

In a further aspect there is provided the use of a composition, which composition comprises an effective amount of an agent which upregulates the level of IFN ϵ , in the manufacture of a medicament for the prophylactic or therapeutic treatment of a condition
30 characterised by a pathogen infection, wherein said medicament is mucosally administered.

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In one embodiment, said pathogen is a microorganism, such as one which is capable of sexual transmission.

5 Reference to “mucosal administration” should therefore be understood as a reference to introducing the subject composition to the mammal by any route which will enable it to contact mucosal immune tissue such as that found, *inter alia*, in the upper respiratory tract, the gastrointestinal tract, the peritoneal cavity, the urinary tract and the reproductive tract. These tissues are commonly termed the gut associated lymphoid tissue (eg. Peyer’s patches
10 in the lamina propria of the small intestine), bronchus associated lymphoid tissue (eg. the diffuse association of lymphocytes, macrophages and other accessory cells found at the airway branches), nasopharyngeal-associated lymphoid tissue (eg. tonsils, adenoids and submucosal lymphoid follicles) and the appendix. Typically, this can be achieved by administering the composition via an intraperitoneal, subcutaneous, intravaginal, oral or
15 inhaled route. In accordance with the exemplification provided herein, said composition is preferably administered via the airways or intravaginally. Although said mucosal administration will generally take the form of administration directly to mucosal tissue, the present invention nevertheless extends to other modes of administration (such as systemic delivery of IFN ϵ) which nevertheless may achieve delivery of the IFN ϵ to mucosa, such as
20 via the vasculature.

Reference to “elicit or induce an immune response” should be understood as a reference to stimulating or facilitating the stimulation of an innate immune response. This innate immune response may then lead to the onset of a specific immune response which is
25 specifically directed to the pathogen in issue.

An “effective amount” means an amount necessary at least partly to attain the desired immune response, or to prevent or to delay the onset or inhibit progression or halt altogether, the onset or progression of a particular condition being treated. This amount
30 varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of the individual to be treated, the capacity of the individual’s immune

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system to stimulate a specific immune response, the degree of protection desired, the formulation of the vaccine, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

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According to this embodiment there is provided a method of eliciting or inducing, in a female mammal exposed to a microorganism, an innate mucosal immune response, said method comprising the mucosal administration of an effective amount of composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .

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In a related aspect there is provided a method for the prophylactic and/or therapeutic treatment of a condition characterised by a microorganism infection in a mammal, said method comprising the mucosal administration of an effective amount of composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .

15

In a further aspect there is provided the use of a composition, which composition comprises an effective amount of an agent which upregulates the level of IFN ϵ , in the manufacture of a medicament for the prophylactic or therapeutic treatment of a condition characterised by a microorganism infection, wherein said medicament is mucosally administered.

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In another embodiment, said microorganism is one which is capable of sexual transmission, in particular herpes simplex virus, human immunodeficiency virus, human papilloma virus or chlamydia. In terms of this embodiment of the invention, the levels of IFN ϵ in the reproductive tract tissue are sought to be increased.

25

It should be appreciated that the method of this aspect of the present invention is directed to effecting an innate immune response to a pathogen, such as a microorganism. Accordingly, the prophylactic aspects of this invention are envisaged to function by upregulating and maintaining elevated IFN ϵ levels in the mucosal tissue of female mammals thought to be at risk of pathogen infection either due to the environment to

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which they have been exposed or because they exhibit abnormally low levels of IFN ϵ . In this case, these individuals will be better placed to respond with a normal innate immune response in the event of their infection with a pathogen. Alternatively, in individuals who have already been exposed to a pathogen, the method of the present invention provides a
5 valuable means of ensuring that an effective innate immune response is generated. In individuals who exhibit reduced levels of IFN ϵ , restoration of IFN ϵ can effect normal innate immune responsiveness. However, even in normal individuals, certain pathogens have developed mechanisms for blocking or limiting normal IFN α and/or IFN β production, thereby limiting pathogen-induced immune responsiveness. Exogenous
10 administration of IFN α and/or IFN β is often associated with significant unwanted side effects. In these situations, whether it be in the context of therapeutic treatment of an existing infection or in the context of achieving an adjuvant effect with a vaccine, upregulation of IFN ϵ levels provides a means to bypass this type of microorganism directed blockade of innate immunity.

15

In accordance with the present invention, there is provided a method of eliciting or inducing an immune response directed to a pathogen, said method comprising the administration of an effective amount of a composition wherein said composition comprises an agent which upregulates the level of IFN ϵ together with one or more
20 pathogen derived antigens.

25

In a further aspect there is provided the use of a composition in the manufacture of a vaccine, which composition comprises an effective amount of an agent which upregulates the level of IFN ϵ together with one or more pathogen derived antigens.

In one embodiment, said pathogen is a microorganism.

Still another aspect of the present invention is directed to a composition suitable for inducing a mucosal immune response to a pathogen, said composition comprising an agent
30 which upregulates the level of IFN ϵ together with one or more pathogen derived antigens.

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In yet another aspect there is provided a vaccine suitable for inducing a mucosal immune response to a pathogen, said vaccine comprising an agent which upregulates the level of IFN ϵ together with one or more pathogen derived antigens.

- 5 In one embodiment, said pathogen is a microorganism, such as a microorganism which is capable of sexual transmission.

The pathogen derived "antigen" may be derived from any source. For example, it may be derived from a naturally occurring, recombinantly produced or synthetically generated
10 polypeptide or protein which, upon uptake by a cell, is subject to processing and presentation of the peptide components derived therefrom in the context of either MHC class I or class II. Alternatively, the antigen may be one which is not derived from a larger molecule but which, in the first instance, takes the form of a peptide. That the antigen is "derived" from the subject pathogen should be understood to mean that the antigen has
15 arisen from the pathogen but has not necessarily been obtained directly from that source. For example, it may have been recombinantly produced or synthetically generated.

Still further, it would be appreciated by the person of skill in the art that the subject antigen is one which generates an immune response that can target the pathogen of interest.
20 Accordingly, the antigen need not necessarily be precisely identical to the corresponding region of the pathogen. For example, minor changes in peptide sequence, such as substitution with conserved amino acids or alteration to glycosylation patterns, may not affect the quality and effectiveness of the immune response generated thereto. The notion of using antigens which, although slightly structurally different to the equivalent region of
25 the pathogen of interest, can induce an immune response which can crossreact with the pathogen of interest in an approach well known to immunologists and commonly used.

The antigen which is utilised in accordance with the method of the present invention may therefore take any suitable form. For example, the antigen may be glycosylated or un-
30 glycosylated, phosphorylated or dephosphorylated to various degrees and/or may contain a range of other proteinaceous or non-proteinaceous molecules fused, linked, bound or

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otherwise associated with the antigen such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins.

It should also be understood that said “antigen” may correspond to a homogeneous
5 population of molecules, (such as a population of recombinantly produced peptides), or it may correspond to a heterogeneous population of molecules such as a sample or fraction of a bacterial culture or attenuated, purified bacteria or parts thereof.

As detailed hereinbefore, the microorganism may be any microorganism, in particular any
10 bacterium, virus, fungus or parasite which can infect a mammal. Reference to a microorganism derived antigen should be understood to extend to any molecule which is secreted by or shed from the subject organism. This would include, for example, toxin molecules or molecules which are cleared from the surface of the microorganism.

15 Reference to an “antigen” should be understood as a reference to any proteinaceous or non-proteinaceous molecule against which it is sought to generate an immune response. To this end, it should be appreciated that, in isolation, the antigen may be immunogenic or non-immunogenic. Without limiting the present invention to any one theory or mode of action, one of the advantages of the development of the method of the present invention is
20 the fact that antigens which are not particularly immunogenic can be made effective as an immunogen when used in accordance with the methods described herein, wherein the IFN ϵ effectively functions as an adjuvant. It should be understood that said immune response will initially take the form of an innate immune response, but may progress to a specific immune response, specifically a B cell and/or T cell response.

25

Yet another aspect of the present invention is directed to a method of preventing implantation of a zygote into the endometrium of a mammal or effecting abortion of an implanted zygote or embryo from the uterus said method comprising administering an agent which upregulates the level of IFN ϵ in the reproductive tract of said mammal.

30

In terms of the agent which upregulates the level of IFN ϵ , this can be any suitable

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molecule including, but not limited to:

- (i) the IFN ϵ protein or functional fragment thereof;
 - 5 (ii) a nucleic acid molecule encoding IFN ϵ or functional fragment thereof;
 - (iii) a molecule which upregulates the expression of IFN ϵ such as by modulating the transcriptional or translational regulation of the IFN ϵ gene.
- 10 The proteinaceous molecules described above may be derived from any suitable source such as natural, recombinant or synthetic sources and includes fusion proteins or molecules which have been identified following, for example, natural product screening. The reference to non-proteinaceous molecules may be, for example, a reference to a nucleic acid molecule or it may be a molecule derived from natural sources, such as for example
- 15 natural product screening, or may be a chemically synthesised molecule. The present invention contemplates analogues of IFN ϵ expression product or small molecules capable of acting as agonists. Chemical agonists may not necessarily be derived from the IFN ϵ expression product but may share certain conformational similarities. Alternatively, chemical agonists may be specifically designed to meet certain physiochemical properties.

20

The proteinaceous and non-proteinaceous molecules referred to in points (i)-(iii), above, are herein collectively referred to as “modulatory agents”.

- Screening for the modulatory agents hereinbefore defined can be achieved by any one of
- 25 several suitable methods including, but in no way limited to, contacting a cell comprising the IFN ϵ gene or functional equivalent or derivative thereof with an agent and screening for the modulation of IFN ϵ protein production or functional activity, modulation of the expression of a nucleic acid molecule encoding IFN ϵ or modulation of the activity or expression of a downstream IFN ϵ cellular target. Detecting such modulation can be
- 30 achieved utilising techniques such as Western blotting, electrophoretic mobility shift

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assays and/or the readout of reporters of IFN ϵ activity such as luciferases, CAT and the like.

It should be understood that the IFN ϵ gene or functional equivalent or derivative thereof
5 may be naturally occurring in the cell which is the subject of testing or it may have been
transfected into a host cell for the purpose of testing. Further, to the extent that an IFN ϵ
nucleic acid molecule is transfected into a cell, that molecule may comprise the entire IFN ϵ
gene or it may merely comprise a portion of the gene such as the portion which regulates
expression of the IFN ϵ product. For example, the IFN ϵ promoter region may be
10 transfected into the cell which is the subject of testing. In this regard, where only the
promoter is utilised, detecting modulation of the activity of the promoter can be achieved,
for example, by ligating the promoter to a reporter gene. For example, the promoter may
be ligated to luciferase or a CAT reporter, the modulation of expression of which gene can
be detected via modulation of fluorescence intensity or CAT reporter activity, respectively.
15 Yet another example includes IFN ϵ binding sites ligated to a minimal reporter.

These methods provide a mechanism for performing high throughput screening of putative
modulatory agents such as the proteinaceous or non-proteinaceous agents comprising
synthetic, combinatorial, chemical and natural libraries. These methods will also facilitate
20 the detection of agents which bind either the IFN ϵ nucleic acid molecule or expression
product itself or which modulate the expression of an upstream molecule, which upstream
molecule subsequently modulates IFN ϵ expression or expression product activity.
Accordingly, these methods provide a mechanism of detecting agents which either directly
or indirectly modulate IFN ϵ expression and/or activity.

25 Without limiting the present invention in any way, IFN ϵ expression is known to be
hormonally regulated. Accordingly, in one embodiment the use of estrogen and estrogen
mimetics provides a useful means of upregulating IFN ϵ levels. In another example, TGF β
can be utilised. Similarly bioinformatic analysis has identified glucocorticoid receptor
30 response elements and Ets factor binding elements within the IFN ϵ promoter. The

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transcription factor binding site BRCA1 has also been identified in the human IFN ϵ promoter. Accordingly, molecules which activate transcription via these sites, such as Elf3 and Elf5, could be utilised to upregulate IFN ϵ expression.

5 The agents which are utilised in accordance with the method of the present invention may take any suitable form. For example, proteinaceous agents may be glycosylated or unglycosylated, phosphorylated or dephosphorylated to various degrees and/or may contain a range of other molecules used, linked, bound or otherwise associated with the proteins such as amino acids, lipid, carbohydrates or other peptides, polypeptides or
10 proteins. Similarly, the subject non-proteinaceous molecules may also take any suitable form. Both the proteinaceous and non-proteinaceous agents herein described may be linked, bound otherwise associated with any other proteinaceous or non-proteinaceous molecules. For example, in one embodiment of the present invention said agent is associated with a molecule which permits its targeting to a localised region.

15 The subject proteinaceous or non-proteinaceous molecule may act either directly or indirectly to modulate the expression of IFN ϵ or the activity of the IFN ϵ expression product. Said molecule acts directly if it associates with the IFN ϵ nucleic acid molecule or expression product to modulate expression or activity, respectively. Said molecule acts
20 indirectly if it associates with a molecule other than the IFN ϵ nucleic acid molecule or expression product which other molecule either directly or indirectly modulates the expression or activity of the IFN ϵ nucleic acid molecule or expression product, respectively. Accordingly, the method of the present invention encompasses the regulation of IFN ϵ nucleic acid molecule expression or expression product activity via the induction
25 of a cascade of regulatory steps.

The term "expression" refers to the transcription and translation of a nucleic acid molecule. Reference to "expression product" is a reference to the product produced from the transcription and translation of a nucleic acid molecule. Reference to "modulation" should
30 be understood as a reference to up-regulation or down-regulation.

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“Derivatives” of the molecules herein described include fragments, parts, portions or variants from either natural or non-natural sources. Non-natural sources include, for example, recombinant or synthetic sources. By “recombinant sources” is meant that the cellular source from which the subject molecule is harvested has been genetically altered.

5 This may occur, for example, in order to increase or otherwise enhance the rate and volume of production by that particular cellular source. Parts or fragments include, for example, active regions of the molecule. Derivatives may be derived from insertion, deletion or substitution of amino acids. Amino acid insertional derivatives include amino and/or carboxylic terminal fusions as well as intrasequence insertions of single or multiple
10 amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in a
15 sequence has been removed and a different residue inserted in its place. Additions to amino acid sequences include fusions with other peptides, polypeptides or proteins, as detailed above.

Derivatives also include fragments having particular epitopes or parts of the entire protein
20 fused to peptides, polypeptides or other proteinaceous or non-proteinaceous molecules. Analogues of the molecules contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecules or their
25 analogues.

Derivatives of nucleic acid sequences which may be utilised in accordance with the method of the present invention may similarly be derived from single or multiple nucleotide substitutions, deletions and/or additions including fusion with other nucleic acid
30 molecules. The derivatives of the nucleic acid molecules utilised in the present invention include oligonucleotides, PCR primers, antisense molecules, molecules suitable for use in

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cosuppression and fusion of nucleic acid molecules. Derivatives of nucleic acid sequences also include degenerate variants.

A “variant” or “mutant” of IFN ϵ should be understood to mean molecules which exhibit at least some of the functional activity of the form of IFN ϵ of which it is a variant or mutant. A variation or mutation may take any form and may be naturally or non-naturally occurring.

A “homologue” is meant that the molecule is derived from a species other than that which is being treated in accordance with the method of the present invention. This may occur, for example, where it is determined that a species other than that which is being treated produces a form of IFN ϵ , for example, which exhibits similar and suitable functional characteristics to that of the IFN ϵ which is naturally produced by the subject undergoing treatment.

Chemical and functional equivalents should be understood as molecules exhibiting any one or more of the functional activities of the subject molecule, which functional equivalents may be derived from any source such as being chemically synthesised or identified via screening processes such as natural product screening. For example chemical or functional equivalents can be designed and/or identified utilising well known methods such as combinatorial chemistry or high throughput screening of recombinant libraries or following natural product screening.

For example, libraries containing small organic molecules may be screened, wherein organic molecules having a large number of specific parent group substitutions are used. A general synthetic scheme may follow published methods (eg., Bunin BA, *et al.* (1994) *Proc. Natl. Acad. Sci. USA*, 91:4708-4712; DeWitt SH, *et al.* (1993) *Proc. Natl. Acad. Sci. USA*, 90:6909-6913). Briefly, at each successive synthetic step, one of a plurality of different selected substituents is added to each of a selected subset of tubes in an array, with the selection of tube subsets being such as to generate all possible permutation of the

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different substituents employed in producing the library. One suitable permutation strategy is outlined in US. Patent No. 5,763,263.

There is currently widespread interest in using combinational libraries of random organic
5 molecules to search for biologically active compounds (see for example U.S. Patent No. 5,763,263). Ligands discovered by screening libraries of this type may be useful in mimicking or blocking natural ligands or interfering with the naturally occurring ligands of a biological target. In the present context, for example, they may be used as a starting point for developing IFN ϵ analogues which exhibit properties such as more potent
10 pharmacological effects. IFN ϵ or a functional part thereof may according to the present invention be used in combination libraries formed by various solid-phase or solution-phase synthetic methods (see for example U.S. Patent No. 5,763,263 and references cited therein). By use of techniques, such as that disclosed in U.S. Patent No. 5,753,187, millions of new chemical and/or biological compounds may be routinely screened in less
15 than a few weeks. Of the large number of compounds identified, only those exhibiting appropriate biological activity are further analysed.

With respect to high throughput library screening methods, oligomeric or small-molecule library compounds capable of interacting specifically with a selected biological agent, such
20 as a biomolecule, a macromolecule complex, or cell, are screened utilising a combinational library device which is easily chosen by the person of skill in the art from the range of well-known methods, such as those described above. In such a method, each member of the library is screened for its ability to interact specifically with the selected agent. In practising the method, a biological agent is drawn into compound-containing tubes and
25 allowed to interact with the individual library compound in each tube. The interaction is designed to produce a detectable signal that can be used to monitor the presence of the desired interaction. Preferably, the biological agent is present in an aqueous solution and further conditions are adapted depending on the desired interaction. Detection may be performed for example by any well-known functional or non-functional based method for
30 the detection of substances.

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Analogues of IFN ϵ contemplated herein include, but are not limited to, modifications to side chains, incorporating unnatural amino acids and/or derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the analogues. The specific form which such
5 modifications can take will depend on whether the subject molecule is proteinaceous or non-proteinaceous. The nature and/or suitability of a particular modification can be routinely determined by the person of skill in the art.

For example, examples of side chain modifications contemplated by the present invention
10 include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH₄; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and
15 pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH₄.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.
20

The carboxyl group may be modified by carbodiimide activation via O-acylisourea formation followed by subsequent derivatisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with
25 iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at
30 alkaline pH.

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Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

5

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carboethoxylation with diethylpyrocarbonate.

- 10 Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids contemplated
- 15 herein is shown in Table 1.

TABLE 1

	Non-conventional amino acid	Code	Non-conventional amino acid	Code
5	α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
	α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
	aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
	aminoisobutyric acid	Aib	L-N-methylaspartic acid	Nmasp
10	aminonorbornyl- carboxylate	Norb	L-N-methylcysteine	Nmcys
	cyclohexylalanine	Chexa	L-N-methylglutamine	Nmgln
	cyclopentylalanine	Cpen	L-N-methylglutamic acid	Nmglu
	D-alanine	Dal	L-N-methylhistidine	Nmhis
15	D-arginine	Darg	L-N-methylisoleucine	Nmile
	D-aspartic acid	Das	L-N-methylleucine	Nmleu
	D-cysteine	Dcys	L-N-methyllysine	Nmlys
	D-glutamine	Dgln	L-N-methylmethionine	Nmmet
	D-glutamic acid	Dglu	L-N-methylnorleucine	Nmnle
20	D-histidine	Dhis	L-N-methylnorvaline	Nmnva
	D-isoleucine	Dile	L-N-methylornithine	Nmorn
	D-leucine	Dleu	L-N-methylphenylalanine	Nmphe
	D-lysine	Dlys	L-N-methylproline	Nmpro
	D-methionine	Dmet	L-N-methylserine	Nmser
25	D-ornithine	Dorn	L-N-methylthreonine	Nmthr
	D-phenylalanine	Dphe	L-N-methyltryptophan	Nmtrp
	D-proline	Dpro	L-N-methyltyrosine	Nmtyr
	D-serine	Dser	L-N-methylvaline	Nmval
	D-threonine	Dthr	L-N-methylethylglycine	Nmetg
30	D-tryptophan	Dtrp	L-N-methyl-t-butylglycine	Nmtbug
	D-tyrosine	Dtyr	L-norleucine	Nle
	D-valine	Dval	L-norvaline	Nva
			α -methyl-aminoisobutyrate	Maib
			α -methyl- -aminobutyrate	Mgab

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	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
	D- α -methylassparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylasspartate	Dmasp	α -methylpenicillamine	Mpen
5	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methyllleucine	Dmleu	α -naphthylalanine	Anap
10	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
15	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- α -methylthreonine	Dmthr	N-cycloheptyl glycyne	Nchep
	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D- α -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D- α -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
20	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylassparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylasspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
25	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
	D-N-methyllleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
30	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe

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	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
5	D-N-methyltyrosine	Dnmtyr	N-methyl- α -naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(p-hydroxyphenyl)glycine	Nhtyr
	L-t-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
10	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
	L- α -methylarginine	Marg	L- α -methylasparagine	Masn
	L- α -methylaspartate	Masp	L- α -methyl-t-butylglycine	Mtbug
	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
15	L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
	L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- α -methylleucine	Mleu	L- α -methyllysine	Mlys
	L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
	L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
20	L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
	L- α -methylserine	Mser	L- α -methylthreonine	Mthr
	L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
	L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
	N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
25	carbamylmethyl)glycine		carbamylmethyl)glycine	
	1-carboxy-1-(2,2-diphenyl-Nmbc			
	ethylamino)cyclopropane			

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Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide
5 and another group specific-reactive moiety.

Modulation of said IFN ϵ functional levels may be achieved via the administration of IFN ϵ , a nucleic acid molecule encoding IFN ϵ or an agent which effects modulation of IFN ϵ activity or IFN ϵ gene expression (herein collectively referred to as “modulatory agents”).

10

Administration of a composition of the present invention in the form of a pharmaceutical composition, may be performed by any convenient means. The components of the pharmaceutical composition are contemplated to exhibit therapeutic or prophylactic activity when administered in an amount which depends on the particular case. The
15 variation depends, for example, on the human or animal. A broad range of doses may be applicable. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation.

20

The composition may be administered in a convenient manner such as by the oral, inhaled, intraperitoneal, subcutaneous, suppository routes or implanting (e.g. using slow release molecules). It may also be administered via non-mucosal routes, where appropriate, such as via intravenous or other such routes. The composition may be administered in the form
25 of pharmaceutically acceptable nontoxic salts, such as acid addition salts or metal complexes, e.g. with zinc, iron or the like (which are considered as salts for purposes of this application). Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, tartrate and the like. If the active ingredient is to be administered in tablet form, the tablet
30 may contain a binder such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate.

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The modulatory agents of the invention can be combined with a pharmaceutically acceptable carrier (excipient) to form a pharmacological composition. Pharmaceutically acceptable carriers can contain a physiologically acceptable compound that acts to, e.g., stabilize, or increase or decrease the absorption or clearance rates of the pharmaceutical compositions of the invention. Physiologically acceptable compounds can include, e.g., carbohydrates, such as glucose, sucrose, or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins, compositions that reduce the clearance or hydrolysis of the peptides or polypeptides, or excipients or other stabilizers and/or buffers. Detergents can also used to stabilize or to increase or decrease the absorption of the pharmaceutical composition, including liposomal carriers. Pharmaceutically acceptable carriers and formulations for peptides and polypeptide are known to the skilled artisan and are described in detail in the scientific and patent literature, see e.g., the latest edition of Remington's Pharmaceutical Science, Mack Publishing Company, Easton, Pennsylvania ("Remington's").

Other physiologically acceptable compounds include wetting agents, emulsifying agents, dispersing agents or preservatives which are particularly useful for preventing the growth or action of microorganisms. Various preservatives are well known and include, e.g., phenol and ascorbic acid. One skilled in the art would appreciate that the choice of a pharmaceutically acceptable carrier including a physiologically acceptable compound depends, for example, on the route of administration of the peptide or polypeptide of the invention and on its particular physio-chemical characteristics.

Solid formulations can be used for enteral (oral) administration. They can be formulated as, e.g., pills, tablets, powders or capsules. For solid compositions, conventional nontoxic solid carriers can be used which include, e.g., pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed. A non-solid formulation can also be used for

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enteral administration. The carrier can be selected from various oils including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, and the like. Suitable pharmaceutical excipients include e.g., starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium
5 stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol.

The composition of the invention, when administered orally, can be protected from digestion. This can be accomplished either by complexing the composition with a
10 composition to render it resistant to acidic and enzymatic hydrolysis or by packaging these molecules in an appropriately resistant carrier such as a liposome. Means of protecting compounds from digestion are well known in the art, see, e.g., Fix (1996) *Pharm Res.* 13:1760-1764; Samanen (1996) *J. Pharm. Pharmacol.* 48:119-135; U.S. Patent 5,391,377, describing lipid compositions for oral delivery of therapeutic agents (liposomal delivery is
15 discussed in further detail, *infra*).

The composition of the invention can also be administered in sustained delivery or sustained release mechanisms, which can deliver the formulation internally. For example, biodegradable microspheres or capsules or other biodegradable polymer configurations
20 capable of sustained delivery of a peptide can be included in the formulations of the invention (see, e.g., Putney (1998) *Nat. Biotechnol.* 16:153-157).

For inhalation, the composition of the invention can be delivered using any system known in the art, including dry powder aerosols, liquid delivery systems, air jet nebulisers,
25 propellant systems, and the like. See, e.g., Patton (1998) *Biotechniques* 16:141-143; product and inhalation delivery systems for polypeptide macromolecules by, e.g., Dura Pharmaceuticals (San Diego, CA), Aradigm (Hayward, CA), Aerogen (Santa Clara, CA), Inhale Therapeutic Systems (San Carlos, CA), and the like. For example, the IFN ϵ formulation can be administered in the form of an aerosol or mist. For aerosol
30 administration, the formulation can be supplied in finely divided form along with a surfactant and propellant. In another aspect, the device for delivering the formulation to

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respiratory tissue is an inhaler in which the formulation vaporizes. Other liquid delivery systems include, e.g., air jet nebulisers.

The IFN ϵ will be formulated in pharmaceutically acceptable compositions suitable for pulmonary or respiratory delivery to a patient. Particular formulations include dry
5 powders, liquid solutions or suspensions suitable for nebulisation, and propellant formulations suitable for use in metered dose inhalers (MDI's). The preparation of such formulations is well described in the patent, scientific, and medical literatures, and the following descriptions are intended to be exemplary only.

10

Liquid formulations of IFN ϵ for use in nebuliser systems can include components to enhance or maintain chemical stability, including chelating agents, protease inhibitors, isotonic modifiers, inert gases, and the like.

15 For use in metered dose inhalers, the IFN ϵ of the present invention will be dissolved or suspended in a suitable aerosol propellant, such as a chlorofluorocarbon (CFC) or a hydrofluorocarbon (HFC). Suitable CFC's include trichloromonofluoromethane (propellant 11), dichlorotetrafluoroethane (propellant 114), and dichlorodifluoromethane (propellant 12). Suitable HFC's include tetrafluoroethane (HFC-134a) and heptafluoropropane
20 (HFC-227).

Preferably, for incorporation into the aerosol propellant, the IFN ϵ of the present invention will be processed into respirable particles as described below for the dry powder formulations. The particles are then suspended in the propellant, typically being coated
25 with a surfactant to enhance their dispersion. Suitable surfactants include oleic acid, sorbitan trioleate, and various long chain diglycerides and phospholipids.

Such aerosol propellant formulations may further include a lower alcohol, such as ethanol (up to 30% by weight) and other additives to maintain or enhance chemical stability and
30 physiological acceptability.

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- Dry powder formulations will typically comprise the IFN ϵ in a dry, usually lyophilized, form with a particular size within a preferred range for deposition within the alveolar region of the lung. Respirable powders of IFN ϵ within the preferred size range can be produced by a variety of conventional techniques, such as jet-milling, spray-drying, solvent precipitation, and the like. Dry powders can then be administered to the patient in conventional dry powder inhalers (DPI's) that use the inspiratory breath through the device to disperse the powder or in air-assisted devices that use an external power source to disperse the powder into an aerosol cloud.
- Dry powder devices typically require a powder mass in the range from about 1 mg to 10 mg to produce a single aerosolized dose ("puff"). Since the required dose of IFN ϵ may be lower than this amount, the IFN ϵ may be combined with a pharmaceutically acceptable dry bulking powder. Preferred dry bulking powders include sucrose, lactose, trehalose, human serum albumin (HSA), and glycine. Other suitable dry bulking powders include cellobiose, dextrans, maltotriose, pectin, sodium citrate, sodium ascorbate, mannitol, and the like. Typically, suitable buffers and salts may be used to stabilize the IFN ϵ in solution prior to particle formation. Suitable buffers include phosphate, citrate, acetate, and tris-HCl, typically at concentrations from about 5 mM to 50 mM. Suitable salts include sodium chloride, sodium carbonate, calcium chloride, and the like. Other additives, such as chelating agents, peptidase inhibitors, and the like, which would facilitate the biological activity of the IFN ϵ once it is dissolved within the lung would be appropriate. For example, ethylenediaminetetraacetic acid (EDTA) would be useful as a chelator for divalent cations which are peptidase cofactors.
- In preparing pharmaceuticals of the present invention, a variety of formulation modifications can be used and manipulated to alter pharmacokinetics and biodistribution. A number of methods for altering pharmacokinetics and biodistribution are known to one of ordinary skill in the art. Examples of such methods include protection of the compositions of the invention in vesicles composed of substances such as proteins, lipids (for example, liposomes, see below), carbohydrates, or synthetic polymers (discussed above). For a general discussion of pharmacokinetics, see, e.g., Remington's, Chapters 37-

39.

The pharmaceutical compositions of the invention can be administered in a variety of unit dosage forms depending upon the method of administration. Dosages for typical
5 modulatory pharmaceutical compositions are well known to those of skill in the art. Such dosages are typically advisory in nature and are adjusted depending on the particular therapeutic context, patient tolerance, etc. The amount of modulatory agent adequate to accomplish this is defined as a "therapeutically effective dose." The dosage schedule and amounts effective for this use, i.e., the "dosing regimen," will depend upon a variety of
10 factors, including the stage of the disease or condition, the severity of the disease or condition, the general state of the patient's health, the patient's physical status, age, pharmaceutical formulation and concentration of active agent, and the like. In calculating the dosage regimen for a patient, the mode of administration also is taken into consideration. The dosage regimen must also take into consideration the pharmacokinetics,
15 i.e., the pharmaceutical composition's rate of absorption, bioavailability, metabolism, clearance, and the like. See, e.g., the latest Remington's; Egleton (1997) "Bioavailability and transport of peptides and peptide drugs into the brain" *Peptides* 18:1431-1439; Langer (1990) *Science* 249:1527-1533.

20 The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion or may be in the form of a cream or other form suitable for topical application. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of
25 microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion
30 and by the use of surfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens,

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chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and
5 gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions
10 are prepared by incorporating the various sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired
15 ingredient from previously sterile-filtered solution thereof.

In one preferred embodiment, modulation of the expression of IFN ϵ is achieved by directly effecting expression of IFN ϵ . Preferably, the introduction of a construct with the gene comprising IFN ϵ will allow for modulation of the levels of IFN ϵ upon expression and
20 thereby effect the biological functions for which it is directed.

Without limiting the present invention to any one theory or mode of action, any cell can accept a gene or gene construct encoding IFN ϵ . However, ideally, the cell can readily accept a gene construct and fully integrate it into the cell to have an influence on the
25 biological function or its own function as well as adjoining cells and cellular environment.

The gene for IFN ϵ may be obtained by PCR amplification of mRNA from human (or other species) tissues using IFN ϵ specific primers and inserted into a mammalian expression vector such as pCDNA3.1 (Clontech) to form a construct or vector that may be
30 transfected into the cell to express IFN ϵ .

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Preferably, a gene sequence for IFN ϵ is operably linked to a regulatory sequence which is capable of providing for the expression of the coding sequence by a cell. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence

5 "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. While operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements e.g. repressor genes may not be contiguously linked to the coding sequence but may still control transcription/translation of the coding sequence.

10

The term "regulatory sequence(s)" includes promoters and enhancers and other expression regulation signals. These may be selected to be compatible with the cell for which the expression vector is designed. Mammalian promoters, such as β -actin promoters and the myosin light chain promoter may be used. However, other promoters may be adopted to

15 achieve the same effect. These alternate promoters are generally familiar to the skilled addressee. Mammalian promoters also include the metallothionein promoter which can upregulate expression in response to heavy metals such as cadmium and is thus an inducible promoter. Tissue-specific promoters may be used. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MML V L

20 TR), the promoter rous sarcoma virus (RSV) L TR promoter, the SV40 promoter, the human cytomegalovirus (CMV) IE promoter, herpes simplex virus promoters or adenovirus promoters. All these promoters are readily available in the art.

Such vectors may be transfected into a suitable cell in which the biological function is

25 desired to provide for expression of a polypeptide encoding IFN ϵ which can then regulate the occurrence of mucosal innate immune responsiveness.

The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of IFN ϵ and optionally a regulator

30 of the promoter. The vectors may contain one or more selectable marker genes for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin

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resistance gene for a mammalian vector. Vectors may be used *in vitro*, for example for the production of RNA or used to transfect or transform a host cell. The vector may also be adapted to be used *in vivo*.

- 5 The cells in which the vector is transfected are expected to provide for such post-translational modifications as may be needed to confer optimal biological activity on the recombinant IFN ϵ .

The vector may be transfected into the cell by any means available to the skilled addressee.

- 10 Preferably, the vector is introduced by calcium phosphate precipitation, electroporation, biolistics, lipofection, naked DNA, DEAE Dextran or adenoviral or retroviral infection. However, this invention is not restricted to these methods.

- If the vector is to be introduced into a germ line to establish a transgenic line, then the transgene may be introduced using anyone of, but not limited to, i) pronuclear microinjection of DNA into a zygote; ii) transfection of preimplantation embryos with recombinant retroviruses carrying the gene of interest; iii) gene transfer into embryonic stem cells by using calcium phosphate-mediated DNA transformation, electroporation, retroviral infection or lipofection; iv) intracytoplasmic coinjection of unfertilized mouse oocytes with exogenous DNA and sperm heads whose membranes had been disrupted. Preferably, pronuclear microinjection is adopted.
- 15
20

- Modulation of IFN ϵ to modulate innate immunity events may be achieved by inducing expression of IFN ϵ by transfection of a construct containing IFN ϵ under the influence of a promoter or by overexpressing the gene in the cell. By introduction of exogenous IFN ϵ or a construct to express exogenous IFN ϵ , the ability of IFN ϵ to modulate mucosal innate immunity may be achieved.
- 25

- The cells are preferably transfected with IFN ϵ by any means that introduces the IFN ϵ gene to the cell. Preferably, the gene encoding IFN ϵ is transfected into the cell via an
- 30

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expression vector by methods routinely available to the skilled addressee or as described above.

Preferably a construct of IFN ϵ is introduced or transfected into the cell to increase the
5 expression the of IFN ϵ . Increasing the expression may be achieved by any means known
to the skilled addressee including the induction of promoters in the construct. Vectors may
be used with regulatory regions that respond to tetracycline, mifepristone or ecdysone.

However, the expression and/or activity of IFN ϵ may also be increased by indirect
10 methods of targeting including regulators to upregulate gene expression. These regulators
may act on the promoters that cause expression of the gene.

Regulation of IFN ϵ gene expression may generally be achieved by the use of molecules
reacting with the promoter of the gene or with a promoter of a nuclear factor regulating the
15 gene, or by RNA processing including splicing and degradation. The activity of proteins
themselves may also be targeted by phosphorylation, or allosteric regulation or regulation
of the protein degradation such as by the use of protease inhibitors.

Increased expression and/or activity of IFN ϵ may be achieved by any means that can
20 increase endogenous IFN ϵ expression and/or activity.

The present invention is further described by reference to the following non-limiting
examples.

25

EXAMPLE 1

Material and Methods

5 *DNA constructs:*

The murine IFN- ϵ , Ifn- β and Ifn- α 4 luciferase reporter construct containing the ATP to - 1000 nucleotides of the IFN- ϵ , Ifn- β and Ifn- α 4 promoter were cloned into the luciferase-reporter plasmid, promoterless pFL3 Basic vector (Promega, Corporation, Madison, WI, 10 USA), respectively. The Irf3 and Irf7 expression plasmids were constructed in the expression plasmid, pCMVs port (Life Technologies Inc., Rockville, MD, USA) which is driven by the cytomegalovirus promoter. The Renilla luciferase reporter plasmid (Promega) is driven by the thymidine kinase promoter and was used as an internal control in transfection experiments.

15

Cells lines:

The murine macrophage cell line, RAW264.7, was grown in complete RPMI (GibcoBRL, Ontario, Canada) consisting of 10% fetal calf serum (FCS; GibcoBRL), 10% L-Glutamine 20 (GibcoBRL) and 1% (v/v) penicillin/streptomycin (GibcoBRL). The human endometrial cell line, Hec1A was grown in complete DMEM/F12 supplemented with 10% FCS (GibcoBRL), 10% L-Glutamine (GibcoBRL) and 1% (v/v) penicillin/streptomycin (GibcoBRL). The human embryonic kidney cell line, HEK293 and green monkey cells, Vero, were grown in complete DMEM/F12 supplemented with 10% FCS (GibcoBRL), 25 10% L-Glutamine (GibcoBRL) and 1% (v/v) penicillin/streptomycin (GibcoBRL). All cell lines were maintained at 37°C in an atmosphere of 5%CO₂.

Transient Transfection and Reporter Gene Assays:

30 HEK293 cells (2 x 10⁴) were plated in a 96 well plate 24 hr prior to co-transfection with 80ng of IFN ϵ or IFN β or IFN α promoter reporter with different irf3, irf7 and irf5

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expression vectors using FuGENE6 (Roche Diagnostics). TK Renilla was used to normalize for transfection efficiency and an appropriate pEF-BOS empty vector plasmid to maintain a constant amount of DNA. Transfected cells were lysed using Reporter Lysis Buffer (Promega) and assayed for luciferase and Renilla activity using luciferase assay
5 reagent (Promega) and Renilla substrate. Luminescence readings were detected using FLUOstar Optima (BMG Technologies). The luminescence readings for Renilla was corrected and expressed as fold induction over empty vector control values.

PAMPs treatment

10

RAW264.7 cells (2×10^6) were seeded in 10cm Petri dish in complete media 24 hr before stimulation. These cells were then stimulated with endotoxin free water to TLR ligands: LPS, 1 $\mu\text{g/ml}$ (Sigma); Pam3Cys, 1 $\mu\text{g/ml}$ (EMC Microcollections GmbH, Tübingen, Germany); poly(I:C), 25 $\mu\text{g/ml}$ (Amersham/GE Healthcare); Cpg oligonucleotide, 1 μM
15 (Gene works, Australia); Loxoribine, 100 μM (Invitrogen). They were then incubated at 37°C for 3 hr.

Generation of $\text{Ifn-}\epsilon^{-/-}$:

20 *Breeding Test:*

3 females KO mice were bred with WT males and vice versa at the age of 6 to 8 weeks. After delivery, the number and sex of the offspring were determined and the litters were inspected daily. The ability of the females to nurse their offspring was monitored, and the
25 weights of pups were measured at 3 weeks, 4 weeks, 5 weeks, 6 weeks of age.

Chlamydia infection:

Female (6 to 8 weeks of age) C57BL/6 wild-type ($\text{Ifn-}\epsilon^{+/+}$) and $\text{Ifn-}\epsilon$ deficient mice ($\text{Ifn-}\epsilon^{-/-}$)
30 $^{-/-}$) were used in these experiments. Seven days following a subcutaneous injection of Progesterone, mice were anaesthetized and challenged intravaginally with Chlamydia

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muridarum. Infection was allowed to progress for 30 days. Mice were monitored on a daily basis to assess body weight, signs of disease, and survival. Disease scores were assigned based on swelling, redness, mucus around the vaginal area and coat. Clearance of chlamydial infection was monitored by the collection of vaginal swabs at day 4, 7, 14, 21 and 29. Swabs were placed into a sterile eppendorf tube and then stored at -80°C . Vaginal lavages were obtained from Chlamydia infected mice at day 1 and 3. Bacterial recovery was assessed using qRT-PCR.

HSV-2 infection:

Female (6 to 8 weeks of age) C57BL/6 wild-type (Ifn- ϵ +/+) and Ifn- ϵ deficient mice (Ifn- ϵ -/-) were used in these experiments. Five days following a subcutaneous injection of 2.0mg DepoProvera, mice were intra-vaginally infected with HSV-2 strain 186 (10000 PFU in 10 μ l). After challenge, mice were monitored on a daily basis to assess body weight, signs of disease, and survival. Mice were weighed individually, and the mean change from initial body weight was calculated daily for each group. Disease scores were assigned based on the following scale: 0, no apparent signs of disease; 1, slight redness of the genitals; 2, swelling and redness of the genitals; 3, mucus, swelling and redness with lesions on the genitals; 4, dead.

Viral plaque assay:

Tissues (vaginal, spinal cord and brain stem) were obtained from HSV-2 infected mice and homogenized using tissue homogenizer. Supernatants were clarified (1500 rpm for 5 mins) and subsequently assessed for virus content by plaque assay on Vero cells as previously described (Carr *et al. Immunology*, 2006. 118(4):520-6). Results are reported in PFU/g tissue.

qRT-PCR:

Total cellular RNA was extracted using TRIzol Reagent (Invitrogen) according to

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manufacturer's instructions and cDNA was synthesized with random hexamer primers (Promega) and M-MLV Reverse Transcriptase (Invitrogen). PCR was performed on an Applied Biosystems 7700 Prism real-time PCR machine with the manufacturer's instructions (Applied Biosystems). Predeveloped TaqMan probe/primers for Ifn- ϵ , Ifn- β ,
5 Isg15, 2'5' oas, Irgm1, Irf7 (Applied Biosystems) were used for calculation of the threshold cycle numbers that were transformed with the cycle threshold and relative value method as described by the manufacturer and were expressed relative to 18S ribosomal RNA. Results are expressed as relative gene expression for each target gene.

10 *Histological analysis:*

Uteri were fixed overnight in 4% paraformaldehyde, then washed in 70% ethanol, and embedded in paraffin. Tissue was sectioned at 4- μ m thickness and stained with H&E.

15 *Determination of estrus cycle:*

The estrus stage of mice were determined from analysis of vaginal smears taken by an autoclaved cotton applicator. The cotton applicator was moistened with PBS prior to take the vaginal swab. The vaginal smears was subsequently stained with Diff-Quick Stain
20 according to the manufacturer's instructions. Stained cells were carefully examined and the estrus stage of each mouse was identified as diestrus, proestrus, early estrus, estrus and postestrus according to a previously established protocol. (Green, E.L. 1989. Biology of the laboratory mouse, 2nd edition. Dover Publications, new York, N.Y.)

25 *Immunophenotyping:*

Single cell suspensions were obtained from Bone marrow, Spleen and Thymus of Ifn- ϵ +/+ and Ifn- ϵ -/- were studied for surface antigen expression using a panel of monoclonal antibodies directly conjugated with fluorochromes. Each tube contained 1 x 10⁶ nucleated
30 cells after adjustment. In order to prevent non-specific binding, cell surface receptors were blocked with Anti-mouse CD16/CD32 Fc γ III/II Receptor blocking antibody (BD

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PharMingen, California). For surface staining, 1×10^6 cells per sample were stained with the various combinations of fluorochrome-labelled antibodies: PE conjugated CD3, Pacific Blue conjugated anti-mouse CD4, APC-Cy7 conjugated anti-mouse CD8, PE-Cy7 conjugated anti-mouse IgM, PE conjugated anti-mouse IgD (BD PharMingen, California).

5 Cells were analysed using a MoFlo High Performance flow cytometer (DakoCytomation, Denmark) and Flo-Jo software.

Results

10 *Regulation of Ifn- ϵ expression.*

Since *Ifn ϵ* is located in the type I IFN locus with all other type I IFN genes, such as *Ifn- β* and *Ifn- α* subtypes which are induced in response to pathogens via PRRs, *Ifn- ϵ* was tested to determine whether it is induced by similar pathways. Raw 264.7 cells, treated with

15 synthetic ligands for TLRs 2,3,4 7/8 and 9 induced known responses genes such as *Ifn- β* and/or *il6*. By contrast, there was no significant change in the expression of *Ifn- ϵ* upon stimulation with these TLR ligands. Besides single TLR ligand stimulation, the regulation of *ifn- ϵ* expression by virus was investigated. Semliki Forest Virus (SFV) infection of Raw264.7 stimulated the expression of the positive control antiviral response genes 2'5'

20 *oas*, but *ifn- ϵ* expression was again unaltered (Figure 1), consistent with the lack of induction by synthetic TLR ligands. Furthermore these results were replicated in epithelial cell line, HEC1A.

Since all PRR including TLRs, RIG-I, STING and AIM2 all induce type I IFN expression

25 through activation of IRF family of transcription factors (Honda *et al. Int Immunol*, 2005, 17(11):1367-78; Onomoto *et al. Curr Top Microbiol Immunol*, 2007. 316:193-205), IRFs were examined to determine whether they could directly regulate the *Ifn- ϵ* promoter. Transient transfection luciferase assay were used. After 24 hrs of transfection in HEC 293 cells, IRF3, IRF7 and IRF5 induced the promoter activity of *Ifn- β* , *Ifn- α* and *p125* (an

30 IRF-binding-site-containing promoter-driven reporter gene containing the wild-type promoter sequence from the *Ifn- β* gene) luciferase reporters, respectively (Sato *et al.*

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Immunity, 2000. 13(4):539-48). However, no significant alteration of the *Ifn-ε* promoter was observed. Thus the data shows that IRFs do not activate the promoter of *Ifn-ε* in a direct manner, consistent with the lack of PRR stimulation of *Ifn-ε* described above.

- 5 Since the expression of *Ifn-ε* was not found to be responsive to pathogens, its regulation in different physiological states was investigated. RNA was isolated from a range of mouse organs and expression of type I IFNs α , β and ϵ by quantitative RT-PCR was measured. The levels of expression of *Ifns* α and β were below reliable detection limits (Figure 2). The expression of *Ifn-ε* mostly followed the same pattern; except for the notable exception
- 10 of the female reproductive tract. *Ifn-ε* is highly expressed in the uterus (~1000 fold above detectable levels) and to a lesser extent, ovary (~100 fold above detectable levels) (Figure 3).

Because of this distinct and unique expression pattern in the uterus, the expression of *Ifn-ε*

15 during estrus cycle was further investigated and was found to vary approximately 30-fold across the estrus cycle. It was lowest during diestrus stage then increases to peak levels at estrus (Figure 3B). Since *Ifn-ε* expression was regulated with the hormonal status of the estrus cycle, its expression during pregnancy was examined. Remarkably, *Ifn-ε* expression in the uterus was dramatically reduced at d4.5 of pregnancy coincident with the time of

20 implantation (Figure 3C). The expression of *Ifn-ε* then gradually increased as pregnancy progressed (Figure 3C). In order to determine whether the reduction required the physical presence of the embryo or its products, or was induced by maternal influences, pseudo pregnant mice were also tested. The *Ifn-ε* expression is again reduced in pseudo-pregnant mice at d4.5 (following mating with vasectomised males) (Figure 3C). These results show

25 that the reduction of *Ifn-ε* expression at implantation is due to the hormonal changes in maternal tissues rather than requiring the physical presence of the embryo. The hormonal regulation of *Ifn-ε* expression during the estrus cycle and pregnancy, suggest that this interferon cytokine, with unique features of regulation, could have a physiological function in reproduction or development.

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Generation and characterisation of $Ifn-\epsilon^{-/-}$ mice

Gene targeted mice were generated in order to examine the pathophysiological functions of this novel cytokine. Homologous recombination was used to introduce LoxP sites flanking the IFN ϵ single exon gene to provide the option of “conditional” gene targeting, and FRT -
 5 flanked Neomycin resistance cassette for selection (Figure 4A). Correctly targeted ES cells were confirmed by Southern blot using 5' (5' external probe was hybridised to SacI digested DNA - wild type band is 8.5kb and the targeted band is 10.5kb as expected) and 3' (3' external probe was hybridised to BamHI digested DNA- the wild type band is
 10 10.2kb and the targeted band is 12.2kb) probes (Figure 4B). To Removal of the neomycin resistance cassette was achieved using flp recombinase. These clones were then screened by PCR with the wild type (631bp) and targeted flp'd (842bp) bands detected. Positive clones were selected for microinjection into blastocysts to generate chimeric mice which were then mated to CMV Cre transgenic mice (REF) to generate Heterozygous mice ($Ifn-\epsilon^{+/-}$).
 15 $^{+/-}$).

The $Ifn-\epsilon^{+/-}$ mice were crossed to generate $Ifn-\epsilon^{+/+}$, $Ifn-\epsilon^{+/-}$ and $Ifn-\epsilon^{-/-}$ offspring. (Figure 4C). Ifn ϵ $-/-$ mice were generated and apparently healthy. Genotyping of mice indicated that IFN ϵ $-/-$ mice were born with the expected Mendelian frequency (25%; Table 2), so
 20 there were no indications of developmental abnormalities. In order to demonstrate that the $Ifn-\epsilon^{-/-}$ mice indeed carried null mutations, uterus was tested by qRT-PCR and the lack of $Ifn-\epsilon$ expression confirmed (Figure 4E). To investigate whether $Ifn-\epsilon$ deficiency has subtle effect on uterine development, uterine morphology was examined by histology. However, there were no significant histological differences in the uterus between $Ifn-\epsilon^{+/+}$ and $Ifn-\epsilon^{-/-}$,
 25 suggesting that $Ifn-\epsilon$ does not play any role in uterine development (Figure 5E).

Since $Ifn-\epsilon$ has a unique expression profile and its expression changes during estrus cycle and pregnancy, the next investigation was directed to whether fertility was affected in $Ifn-\epsilon^{-/-}$ mice. $Ifn-\epsilon^{-/-}$ females and males were mated with both $Ifn-\epsilon^{+/+}$ and pairs. There was no
 30 significant difference in the number of offspring nor litter sizes generated by $Ifn-\epsilon^{-/-}$

females or males relative to wild type mice tested over 6 litters for each breeding pair(Figure 4E). There was no difference in litter size between *Ifn-ε*^{+/+} mating and *Ifn-ε*^{-/-} mating (Figure 4E). These data indicate that, despite constitutive and exclusive expression in the female reproductive tract, *Ifn-ε* does not play a non-redundant role in male or
 5 female fertility.

Conventional type I IFNs, regulate the development and activities of immune cells, notably of the myeloid and osteoclasts lineage (Kolumam *et al. J Exp Med*, 2005, 202(5):637-50; Hwang *et al., Proc Natl Acad Sci U S A*, 1995, 92(24):11284-8). Therefore the
 10 development of myeloid, T and B cell lineages in *Ifn-ε*^{-/-} mice was examined by flow cytometric analysis. However, there is no difference detected in myeloid population in bone marrow (Figure 5A) nor T cells and B cell populations in spleen, thymus and bone marrow of *Ifn-ε*^{-/-} mice compared to *Ifn-ε*^{+/+} mice (Figures 5B-D). Thus, the data
 15 demonstrate that *Ifn-ε* is not essential for haemopoiesis of meloid or lymphoid lineage cells.

Ifn-ε signalling in the female reproductive tract.

Conventional type I IFNs transduce signals through the well characterised JAK-STAT
 20 pathway to regulate the expression of groups of up to 2,000 IRGs whose encoded proteins perform the activities ascribed to IFNs such as inhibition viral replication, modulation of cell proliferation, survival or migration. Given that it is constitutively expressed in the uterus, investigation was directed to whether IRGs regulated by *Ifn-ε* would reduce expression without its expression in *Ifn-ε*. null mice. 5 IRGs showed reduction in the
 25 uterus. As shown in Figure 6 (A-D), the levels of expression of several IRGs, namely, ISG15, 2'5' oas, IRF7 and IRGM1, are dramatically reduced in the uteri of *Ifn-ε*^{-/-} mice. In order to confirm this gene reduction is due to the absence of *Ifn-ε*, the expression of these IRGs has also been tested in the kidney, where *Ifn-ε* is not constitutively expressed. As expected, the kidney expression of these IRGs showed no difference between *Ifn-ε*^{+/+}
 30 and *Ifn-ε*^{-/-} in the (Figures 7A-C).

Role of $Ifn-\epsilon$ in protection of FRT from genital Chlamydia infection

IFN ϵ was examined to determine whether it was important in protecting the female
5 reproductive tract from infection with Chlamydia, the most prevalent bacterial STI which
leads to pelvic inflammatory disease and infertility. The susceptibility of $Ifn-\epsilon^{-/-}$ mice to
the well characterised murine model of genital infection *Chlamydia Muridarum* was
evaluated. Following a sublethal dose, mice were monitored by measuring weight loss and
clinical signs (swelling, redness at the vagina, mucus production and coat) for the course of
10 infection. $Ifn-\epsilon^{-/-}$ displayed more severe clinical signs of disease from 7 days of post-
infection, throughout the experiment until 30 days post infection in contrast to $Ifn-\epsilon^{+/+}$
mice which displayed minimal signs of illness at this dose infection(Figure 8A). In
addition, more bacteria were recovered from vaginal swabs throughout the course of
infection (Figure 8B),

15

In order to determine the role of $Ifn\epsilon$ in susceptibility to infection, bacterial recovery from
the uterine wall at early time points in the infection was investigated. By 3 days post-
infection, where the levels of bacteria had not increased from the day 1, there was an
increase in the levels of bacteria in $Ifn-\epsilon^{-/-}$ (Figure 8C). These data demonstrate the
20 importance of the constitutive $Ifn\epsilon$ in the susceptibility of mice to Chlamydia infection.

The role of $Ifn\epsilon$ in clearance of infections was next examined. Whereas by 30 days the
wild type mice had cleared bacteria and no detectable Chlamydia, on the other hand, in
the absence of IFN ϵ , there were significantly high levels of infectious bacteria detected,
25 ranging up to 10^5 (Figure 8D). There were no changes in $Ifn-\epsilon$ RNA expression at the
early or late timepoints post infection (Figure 8E), consistent with data presented earlier
that $Ifn-\epsilon$ is not induced by bacteria, nor TLR ligands. Also the results further highlight the
difference between $Ifn-\epsilon$ and the other type I IFNs which are induced by pathogens
including Chlamydia. Importantly these data clearly demonstrate the importance of
30 constitutive production of IFN ϵ in the female reproductive tract to optimize protection
from or optimal clearance of this bacterial infection.

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Ifn- ϵ ^{-/-} mice are susceptible to genital HSV-2 infection

In order to determine whether this unique, constitutive IFN ϵ is also important in protecting the female reproductive tract from viral infection, the effect of genital HSV-2 infection in *Ifn- ϵ ^{-/-}* mice was examined. Both *Ifn- ϵ ^{+/+}* and *Ifn- ϵ ^{-/-}* mice were challenged with a sublethal dose of HSV-2 and were monitored by measuring weight loss and clinical scores for 7 days. Weight loss was observed in both groups of mice following infection, but by day 6 and 7 post infection, the weight loss was significantly more severe in the IFN ϵ ^{-/-} mice, indicative of more severe disease (Figure 9A). In addition, *Ifn- ϵ ^{-/-}* mice had significantly worse clinical scores of disease relative to wild type mice. Whereas wild-type mice developed only mild symptoms ranging from a clinical score of 0 to 1, *Ifn- ϵ ^{-/-}* mice developed more severe disease by day 4 post infection which worsened to a severity score of 4 (Figure 9B) with severe epidermal lesions evident by that time (Figure 9C). This data was consistent with the elevated viral titers in the infected vagina of *Ifn- ϵ ^{-/-}* mice at day 3 p.i., compared with *Ifn- ϵ ^{+/+}* animals (Figure 9D). These results demonstrate the importance of *Ifn- ϵ* in protecting the female reproductive tract from infection with HSV-2.

EXAMPLE 2

Conventional vaccines mostly contain an adjuvant whose role is to stimulate an innate immune/inflammatory response via TLRs and type I IFN responses. This innate response acts to prime boost the ensuing adaptive immune recall response. Some pathogen infections e.g. HSV-2 are able to evade this response and hence there have not been any successful vaccines developed to this virus. Since conventional vaccines will not boost the specialist anti-infection, immunostimulatory and unconventional IFN ϵ in the reproductive tract, we tested several strategies for IFN ϵ activation in a vaccine, namely –

1. Addition of recombinant mIFN ϵ
2. Addition of a DNA vaccine vector expressing IFN ϵ
3. Addition of anti-progesterone compounds: neutralizing antibody, or progesterone

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receptor antagonist, RU486 to depress endogenous IFN ϵ production

These components, are tested by their inclusion in standard vaccine experiments alongside no adjuvant or a conventional adjuvant (e.g. TLR agonist such as CpG DNA) controls. The effects of treatment with IFN ϵ or its modulators alone as a prevention or therapy (in the absence of immunogen) is also assessed:

An experimental model of HSV infection established using a human clinical isolate of HSV-2 is used. Mice are immunized with attenuated HSV-2 via both local and systemic routes. Either 4 weeks following a single intravaginal immunization or three weekly sc immunizations, mice are infected with the clinical isolate of HSV-2. Disease severity is scored by a clinical scoring system, histological assessments and viral titres. Mucosal immune responses are determined by ELISA measurement of anti-HSV IgG and IgA and immunomodulatory cytokines in serum and vaginal washes. Cellular immune responses are measured by analyzing numbers of effector cells by flow cytometry and their activity by expression of activation markers and cytotoxicity assays of cells isolated from tissues in the RT, blood, draining lymph nodes and spleen. Cells are also harvested from spine and brain stem as HSV-2 infects the CNS where it establishes a latent infection. Therefore the role of IFN ϵ treatment in modulation of viral shedding and inflammation in a chronic established and latent infection is determined.

Modulation of endogenous IFN ϵ levels is monitored by qRT-PCR and immunoassays. Demonstration of increased efficacy occurring via IFN ϵ modulation is achieved by comparison with control experiments using IFN ϵ -/- mice.

The timing of administration of vaccine or IFN ϵ /stimulator is tested to determine whether effects are optimal when the endogenous IFN ϵ levels were suppressed, for example during diestrus stage of cycle, implantation stage of pregnancy or when administering exogenous progestins.

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EXAMPLE 3

Characterisation of acute HSV-2 infection in IFN ϵ ^{-/-} mice compared with WT mice

HSV-2 propagation

5

A clinical isolate of HSV-2 (strain 186) from Prof. Cunningham, was propagated in Vero cell monolayers and viral titres determined by plaque assay.

In vivo infection

10

Female 6-8 week old WT C57BL/6 IFN ϵ ^{-/-} mice are subcutaneously injected with 2mg progesterone 5 days prior to infection with 2,400PFU HSV-2 via intravaginal inoculation. This dose is based on that found to induce a mild disease in WT HSV-2-infected mice, peaking at 5-7 days. Mock-infected WT and IFN ϵ ^{-/-} mice receive PBS. Mice are monitored for survival for 7 and 30 days post-infection and clinical signs of illness are scored for presence of swelling, mucous production, redness and overt lesions: 0—healthy; 1-genital erythema; 2-moderate genital inflammation; 3-genital lesion / generally bad condition; 4—hind-limb paralysis; 5—Moribund/dead.

20 *IFN ϵ in susceptibility*

Dose response experiments are performed to determine a dose of virus which may not induce disease in WT but does in IFN ϵ ^{-/-} mice.

25 *Histology assessment*

Histology is performed on formalin fixed, paraffin embedded tissues including vagina, cervix, uterine horns, oviduct, and ovaries to determine the ascending nature of infection. H&E and PAS (for mucin) stained tissue sections will be assessed for scarring, collagen deposition, cervix/uterus remodelling (myometrium thickness, endometrial thickness and lumen thickness), and oviduct pathology (luminal dilation/diameter, loss of epithelial folds,

30

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reduction in wall thickness) (Wood *et al.* 2007, *Reproduction* 133:1035-1044).

Viral replication

- 5 Viral replication is determined in vaginal washings after 24 and 48h and in tissue samples from the reproductive tract segments described above, and brain stem to track the ascending infection in mice killed after 7 days. HSV-2 titres is determined by plaque assay by a cytopathic effect bioassays to assess TCID₅₀ and PCR for HSV-2 glycoprotein D.
- 10 In order to determine the effect of IFN ϵ on HSV-2 replication in specific cellular compartments, HSV strains with GFP-tagged VP26 gene (no impairment of replication kinetics *in vitro*) are used. Immunofluorescence studies will enable localisation of virus to particular cells.

15 **Influence of hormones on IFN ϵ affects in HSV-2 infection**

- Based on the role of hormones in the susceptibility of women to STIs, the requirement for hormonal pre-treatment in murine models of reproductive tract infection and also the hormonal regulation of IFN ϵ expression, the hormone regulation of HSV-2 susceptibility
- 20 occurs via modulation of IFN ϵ levels. IFN ϵ ^{-/-} mice are used to determine the relationship between IFN ϵ , hormones and HSV-2 infection.

- The stage of estrus cycle is important for initial infection with HSV-2. To assess the separate roles of hormone regulation and IFN ϵ in establishment and progression
- 25 of infection, WT or IFN ϵ ^{-/-} mice at different stages of cycle will be infected with HSV-2 and assessed as above.
- WT and IFN ϵ ^{-/-} mice are untreated or treated with progesterone (2mg medroxyprogesterone acetate in 200 μ l PBS, s.c.) at day -5 as above, then infected
- 30 with HSV-2 (day 0), and monitored as above.

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- Since IFN ϵ levels are highest at estrus when oestrogen levels are highest, the effects of oestrogen (0.5 mg oestradiol benzoate in 200 μ l PBS, s.c.) on HSV-2 infection of WT and IFN ϵ ^{-/-} mice are determined. Entry into and stability of estrus is confirmed by vaginal smear. Seven days after treatment mice are infected with IFN ϵ expression, viral replication, pathology and immunity will be assessed as above.

Analysis of innate immunity

- 10 • IFN ϵ ^{-/-} and WT C57B1/6 mice will be assessed at various time points post HSV-2 infection. Samples of the vagina, cervix, and uterine horns, oviducts and ovaries are collected, fixed and sectioned or cell suspensions made to stain with antibodies for immune cells known to impact on HSV-2 infection, namely NK, monocytes, DC, neutrophils, and lymphocytes.
- 15 • To ascertain the IFN ϵ producing cells and analyse changes that occur over the course of infection, *in situ* hybridisation is performed for gene expression and immunohistochemistry to confirm protein expression and correlate with cells replicating virus.
- Induction of innate immune response genes is measured from above tissues by RT-PCR: TLRs, as they are IRGs and influence the reaction to infection; cytokines, to assess activation of TLR pathways: IL6, TNF α and IFNs α , β ; immunoregulatory cytokines and chemokines: CXCL10 and CXCL9, (type I IFN responsive chemoattractants for monocytes, DC, T, B and NK cells, GMCSF, IFN γ , RANTES, MCP-1, other protective factors such as mucins: Muc1 and antiviral IRGs including PKR and 2'5' OAS.
- 20 • In order to ascertain whether hormones are regulated by IFN ϵ , serum oestrogen and progesterone levels are determined by radioimmunoassay.
- 25

Analysis of adaptive immune response

30

- The inflammatory immune cell infiltrate in infection is defined and enumerated by

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flow cytometry analysis, locally at the site of infection, of vaginal tissue samples and lavage washes, as well as draining iliac and inguinal lymph nodes, and systemically, in spleen, spinal cord and brain stem. Effector (CD8, CD4) populations are assessed as described above and their activation status by surface marker expression. Polarisation and activation of T cells (Th1, Th2, Th17, T regs) is characterised by a combination of surface (CD62L, CD44, CD25) and intracellular cytokine staining (IFN γ , IL-4, IL-17, IL-10 respectively) as described.

- Adaptive cytokines (IFN- γ , IL-12p40) and chemokines (CXCL10, CXCL9, RANTES, KC) produced locally (vaginal lavage) and systemically (serum) are measured at days 3 and 7. Antigen-specific effects of lymphoid cells are determined by *in vitro* restimulation of cells with antigen to HSV (HSV-gB) to assess the effect of IFN ϵ on the effector recall response.
- Serum antibody (IgG)-responses are assessed in mice that survive to 30 days post-infection by direct ELISA. Secretory antibody (IgA) is assessed to confirm previous studies in IgA^{-/-} mice showing that IgA is not required for protection against HSV-2 vaginal infection and also to determine any regulation by IFN ϵ .

20 Analysis of molecular mechanisms

- Microarray studies are performed on tissue from infected (day 1, 3, 5 and 7) versus uninfected WT and IFN ϵ ^{-/-} mice to determine novel gene signatures of activation for IFN ϵ in HSV-2 infection. Experiments are performed using the Agilent platform on 44,000 probes. Initial analysis of data is performed using Genespring program.

Anti-HSV-2 effects of IFN ϵ in vitro.

- 30 Recombinant muIFN ϵ is tested for antiviral activity against HSV-2 using established IFN bioassays initially on generic cells e.g. fibroblasts as representative of connective tissue

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effects and epithelial cells, then isolated primary epithelial cells from uteri of WT and IFN ϵ ^{-/-} mice. The effects of IFN ϵ on cell biology characteristics such as proliferation, survival (after growth factor withdrawal), senescence, migration or differentiation is assessed using standard procedures. These activities of rIFN ϵ are compared to rIFN α and
5 rIFN β , that have been produced similarly.

The effect of exogenous IFN ϵ on HSV-2 infection in vivo

IFN ϵ ^{-/-} mice (naïve) or WT mice (overexpression) receive either direct rmIFN ϵ or IFN ϵ
10 transgene injection. Initially, dose response and timecourse studies of HSV-2 infection are performed and analysed as described above.

To prevent infection:

15 rIFN ϵ is administered intra-vaginally to WT and IFN ϵ ^{-/-} mice every 3 days initially using 1 μ g doses which are effective for other type I IFNs. If this dose is not effective then dose escalation studies would be undertaken. IFN ϵ administration commences 4 days prior to infection (day -4). Controls receive placebo. Studies with IFN ϵ transgene delivery are performed according to published protocols. Mice are infected with HSV-2 and the
20 protective effects of IFN ϵ administration on survival, pathology and immune responses will be assessed as above. Since IFN ϵ is normally well tolerated whereas IFNs α and β display dose-limiting toxicities, side effects including rectal temperature(CIA)³², serum inflammatory markers like U/E, CRP and cytokines will be monitored. Induction of IRGs over the timecourse of infection will verify IFN ϵ activity.

25

To treat infection:

These studies are repeated but treatment will commence at the peak of infection at day 2. Dosage, timing and assessments are performed as described above.

30

Effects of IFN ϵ on HSV-2 infection of human cells

The role of exogenous and endogenous IFN ϵ on human cells

- 5 • Effects of endogenous and exogenous IFN ϵ on cell biology parameters are measured in the aforementioned groups by proliferation and survival apoptosis after growth factor withdrawal.
- 10 • Effects of IFN ϵ on immune parameters are determined by measurement, by RT-PCR of cell lysates, or ELISA of cell supernatants cytokines, chemokines, mucins and defensins, PRRs and IRGs, similar to those described in the murine model studies. Cells are harvested 0, 1, 3, 12, 24, 24 hrs post treatment.
- 15 • IFN ϵ may affect cells influenced by the direction of production from the epithelial cells, either apically into the uterine/vaginal lumen or basally into the tissue. To determine, the directional secretion of and effects, epithelial cells are cultured in transwell inserts until they achieve polarity (measured by electrical resistance) and secreted IFN ϵ levels measured in each direction. Then IFN ϵ is added to cells (from each direction) and products determined in fluids from each “side” as described above.

20 *Direct or indirect effects of IFN ϵ on HSV-2 infection*

- 25 • The aforementioned groups of epithelial cell lines are infected *in vitro* with HSV-2 (strain 186) (3 MOI) for 2 hours. Supernatants are recovered at 24 and 48 hours and stored for viral titration using standard plaque forming assays as described above. Cells are recovered to assess the viral load and selected IRG levels as described above.
- 30 • To assess if constitutive IFN ϵ in the uterine mucosa protects against ascending cervical infection in humans, representative epithelial cell lines above are untreated or treated with siRNA against IFN ϵ or scrambled siRNA. After 24 hours, cells are treated with rIFN ϵ or placebo and 24 hours later are infected and assessed by

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discussed parameters above.

- To assess treatment rIFN ϵ is administered to these cell lines at the same time as or 24 hours after infection.

5 Hormonal regulation of IFN ϵ and HSV-2 infection

Cells are cultured in hormone stripped media +/- progesterone (10^{-6} M) or 17β -oestradiol (10^{-9} M) for 24 hours pre-infection or IFN ϵ treatment and experiments performed as above.

- 10 Once optimised, primary epithelial cells from the ectocervix, endocervix and uterus are then utilised to validate critical data. Cells are obtained (AIs 2 and 5) from healthy donor women by outpatient biopsy (Pipelle), specifically collected for this study. In each case, the stage of the menstrual cycle is classified into early-mid proliferative, late proliferative, early-mid secretory, or late secretory phase on the basis of morphology.

15

EXAMPLE 4

Comparative biological activities of IFN ϵ *in vitro*

- 20 Despite a relatively low (>1000-fold) lower antiviral specific activity *in vitro*, the ability of recombinant murine IFN ϵ to induce interferon response genes (IRGs) that mediate innate immune responses is of equal potency to IFNs $\alpha 1$ and β (Figure 12). Figure 12 also shows that the IRG induction by IFN ϵ is mediated by the Ifnar1 and 2 receptor components that mediate the activities of IFNs α and β (since the genes are not induced in the Ifnar1 and Ifnar2 null cells –bone marrow derived macrophages).

25

EXAMPLE 5

Production and characterisation of recombinant IFN ϵ

Recombinant protein expression:

- 30 Recombinant mouse Interferons were expressed using a baculovirus insect cell expression system. Recombinant baculovirus constructs encoding mIFN $\alpha 1$, mIFN β and mIFN ϵ were

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used to infect High Five cells (Tichoplusia in egg cell homogenate cell line BTI-TN-5B1-4) and left to express protein for 48hrs at 27°C. mIFN α 1 and mIFN β are secreted into the culture media by the gp67 signal peptide, resulting in N-terminally 6xHis tagged proteins, which were purified by immobilising metal affinity chromatography on a Nickel resin, followed by size exclusion chromatography on a Superdex S200 16/60 column. mIFN ϵ is secreted into the culture media by the honeybee mellitin signal peptide and retains its native N-terminus with the addition of two amino acid residues, an alanine and a glutamic acid. mIFN ϵ is purified by immunoaffinity chromatography with an anti-IFN ϵ specific monoclonal antibody generated in our laboratory. The recombinant interferons are shown to be >95% purity by SDS-PAGE Coumassie.

Antiviral specific activity of recombinant IFN ϵ produced in insect cells and purified by chromatography is $2.1 \pm 0.3 \times 10^5$ IU /mg protein (Figure 13). Protein was determined to be > 95% pure and free of detectable endotoxin. This specific activity was determined in a Cytopathic Effect Inhibition assay for bioactivity of an interferon, using murine L cells and Semliki Forest Virus and calibrated against NIH international reference standard for IFN α . This activity is considerably less than that for IFN α which is typically 2×10^8 IU/mg protein.

20

EXAMPLE 6

In vivo immunomodulatory activities of IFN ϵ

Wild-type C57Bl/6 mice were injected either intraperitoneally or intravaginally with 1000IU mIFN α 1 or 1 μ g mIFN ϵ . After three hours, the mice were culled and a peritoneal lavage performed. Additionally, the vagina, uterus, ovaries, kidneys, liver and inguinal lymph nodes were collected for RNA analysis. The spleen and serum were collected for FACS and cytokine analysis, respectively. RNA was extracted from the snap frozen organs using TRIsure and reverse transcribed using MMLV reverse transcriptase. RT-PCR was performed using Applied Biosystems TaqMan at the Gandel Charitable Trust Sequencing Centre.

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IFN ϵ was produced and purified and injected *in vivo* to demonstrate immunomodulatory activities and compared to IFN α . Female 6- week old C57Bl/6 (WT) mice were treated with PBS, rIFN ϵ or IFN α (i.p.) for 3 hours. Spleens were harvested and single cells suspensions prepared. Cells were unstimulated (media) or stimulated for 24hr with
5 100ng/ml repurified LPS. N=3 individual mice per group. Figure 14 demonstrates that IFN ϵ has potent ability to prime immune cells (splenocytes) for optimal innate immune response to LPS (the prototypical proinflammatory stimulus), as measured by the production of IL6 and TNF. While slightly higher dose of IFN ϵ was administered compared to IFN α (1ug for the former vs 0.3 ug of the latter), this immunomodulatory
10 activity was very high in comparison to the antiviral activity of IFN ϵ which was >1000-fold lower than IFN α .

In another experiment IFN ϵ and IFN α in the same amounts as above, were administered i/p (i.e. systemically) to mice and peritoneal cells isolated and tested for changes in the
15 expression of immunoregulatory genes. Figure 15 demonstrates that IFN ϵ was a potent inducer of the potent immunoregulatory IFN γ and ISG15. Interestingly IFN ϵ was even more potent than IFN α in inducing TNF and IL6 and chemokines CCL3 and CCL2.

EXAMPLE 7

20 Activity of IFN ϵ when administered mucosally (intravaginally)

IFN ϵ but not IFN α induced the proinflammatory cytokine TNF α , meaning that IFN ϵ has *in vivo* activity that would not be predicted from comparisons with IFN α and given its very low characteristic antiviral activity, perhaps not even relative to its own activities as
25 determined *in vitro* (Figure 16) CCL2 activation by IFN ϵ showed a trend towards increased activity, but was not statistically significant – may be with more numbers.

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EXAMPLE 8

Immunomodulatory information from IFN ϵ knockout mice

IFN ϵ ^{-/-} mice showed decreased activated macrophages (Mac1⁺ F4/80⁺) in the peritoneal
5 cavity. When administered IFN ϵ , the numbers of these cells increased. This shows that the
novel IFN modulates these important effector cells of innate immunity.

Furthermore, in models of female reproductive tract infection (Chlamydia, HSV), there
was a tendency for increased levels of CD8⁺ (effector) T cells, demonstrating that IFN ϵ
10 modulates adaptive immune cells.

Those skilled in the art will appreciate that the invention described herein is susceptible to
variations and modifications other than those specifically described. It is to be understood
that the invention includes all such variations and modifications. The invention also
15 includes all of the steps, features, compositions and compounds referred to or indicated in
this specification, individually or collectively, and any and all combinations of any two or
more of said steps or features.

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Table 2

Genotype	Wild Type	Heterozygous	Knockout	Total
Number of mice	35	88	41	164
Actual Ratio	1.0	2.2	1.1	
Expected ratio	1	2	1	

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CLAIMS:

1. A method of eliciting or inducing, in a female mammal exposed to a pathogen, a mucosal immune response, said method comprising the administration of an effective amount of a composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .
2. A method for the prophylactic and/or therapeutic treatment of a condition characterised by a pathogen infection in a female mammal, said method comprising the administration of an effective amount of a composition wherein said composition comprises an agent which upregulates the level of IFN ϵ .
3. Use of a composition, which composition comprises an agent which upregulates the level of IFN ϵ , in the manufacture of a medicament for the prophylactic or therapeutic treatment of a condition in a female mammal characterised by a pathogen infection.
4. The method or use of any one of claims 1-3 wherein said immune response is an innate immune response
5. The method or use of any one of claims 1-4 wherein said pathogen is a microorganism.
6. The method or use according to claim 5 wherein said microorganism is capable of sexual transmission.
7. The method or use according to claim 6 wherein said microorganism is herpes simplex virus, human papilloma virus, human immunodeficiency virus or chlamydia trachomatis.
8. The method or use according to any one of claims 1-7 wherein said administration

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is mucosal administration.

9. A method of eliciting or inducing an immune response directed to a pathogen, said method comprising the administration of an effective amount of a composition wherein said composition comprises an agent which upregulates the level of IFN ϵ together with one or more pathogen derived antigens.
10. Use of a composition in the manufacture of a vaccine, which composition comprises an effective amount of an agent which upregulates the level of IFN ϵ together with one or more pathogen derived antigens.
11. A vaccine suitable for inducing a mucosal immune response to a pathogen, said vaccine comprising an agent which upregulates the level of IFN ϵ together with one or more pathogen derived antigens.
12. The method, use or vaccine according to any one of claims 9-11 wherein said pathogen is a microorganism.
13. The method, use or vaccine according to claim 12 wherein said microorganism is capable of sexual transmission.
14. The method, use or vaccine according to claim 13 wherein said microorganism is herpes simplex virus, human papilloma virus, human immunodeficiency virus or chlamydia trachomatis.
15. A method of preventing implantation of a zygote into the endometrium of a mammal or effecting abortion of an implanted zygote or embryo from the uterus said method comprising administering an agent which upregulates the level of IFN ϵ in the reproductive tract of said mammal.
16. The method, use or vaccine of any one of claims 1-15 wherein said agent is the

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IFN ϵ protein or functional fragment thereof.

17. The method, use or vaccine of any one of claims 1-15 wherein said agent is a nucleic acid molecule encoding IFN ϵ or functional fragment thereof.
18. The method, use or vaccine of any one of claims 1-15 wherein said agent is a molecule which upregulates the expression of IFN ϵ .
19. The method, use or vaccine according to claim 18 wherein said agent is estrogen, an estrogen mimetic or TFG β .
20. The method, use or vaccine according to claim 18 wherein said agent is a molecule which activates transcription via the glucocorticoid, Ets or BRCA1 binding sites.
21. The method, use or vaccine according to claim 20 wherein said agent is Elf3 or Elf5.
22. A method of screening for a predisposition to developing a pathogen infection of the reproductive tract in a female mammal said method comprising measuring the level of expression of IFN ϵ in a biological sample from said mammal wherein a lower level of expression of said IFN ϵ relative to control levels is indicative of a predisposition to developing a pathogen infection of the reproductive tract.
23. A method for monitoring a female mammal's predisposition to developing a pathogen infection of the reproductive tract, said method comprising measuring the level of expression of IFN ϵ in a biological sample from said mammal wherein a lower level of expression of said IFN ϵ relative to control levels is indicative of a predisposition to developing a pathogen infection of the reproductive tract.
24. The method according to claim 22 or 23 wherein said pathogen is a microorganism.

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25. The method according to claim 24 wherein said microorganism is capable of sexual transmission.
26. The method according to claim 25 wherein said microorganism is herpes simplex virus, human papilloma virus, human immunodeficiency virus or chlamydia trachomatis.
27. The method according to any one of claims 22-26 wherein said biological sample is a blood sample, vaginal swab or vaginal wash.
28. The method according to any one of claims 22-27 wherein said level of expression of IFN ϵ is the protein level.
29. The method according to any one of claims 22-27 wherein said level of expression of IFN ϵ is the primary RNA or mRNA transcript level.
30. The method according to claim 29 wherein said RNA transcript levels are measured by transcribing said RNA to cDNA.
31. The method, use or virus according to any one of claims 1-30 wherein said mammal is a human.

FIGURE 1A

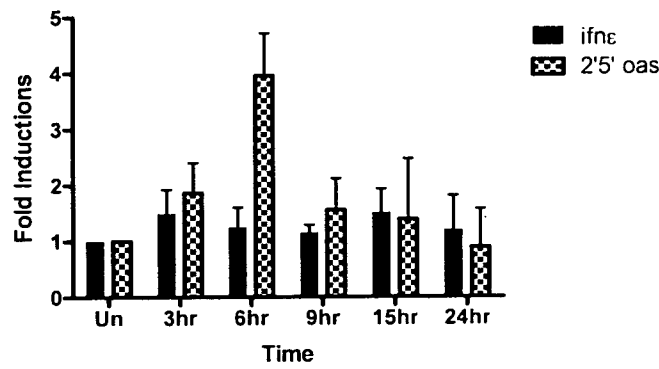


FIGURE 1B

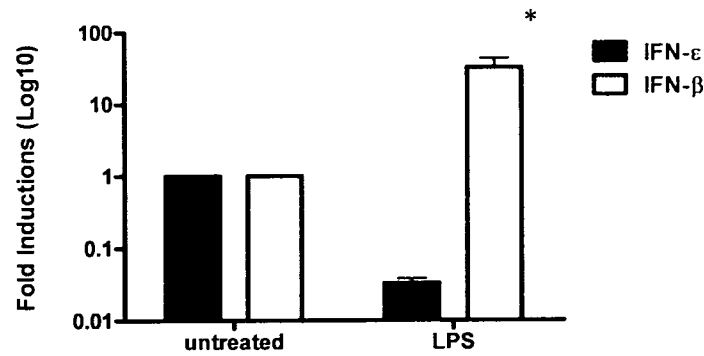


FIGURE 2

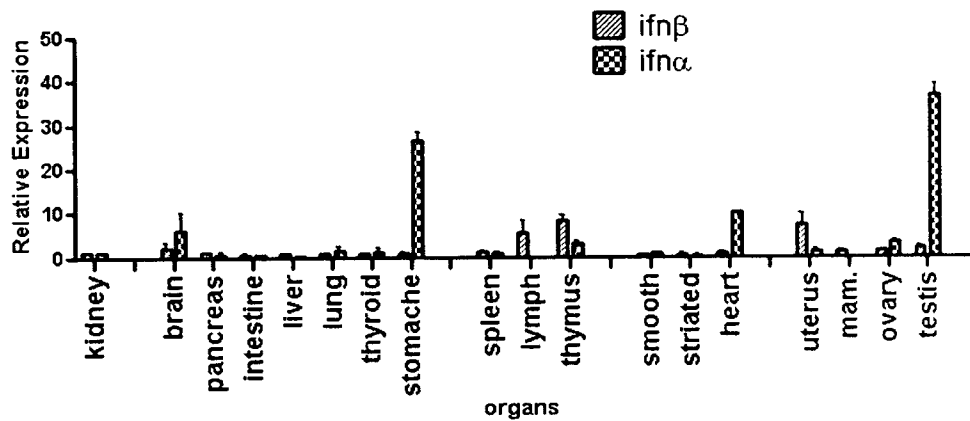


FIGURE 3A

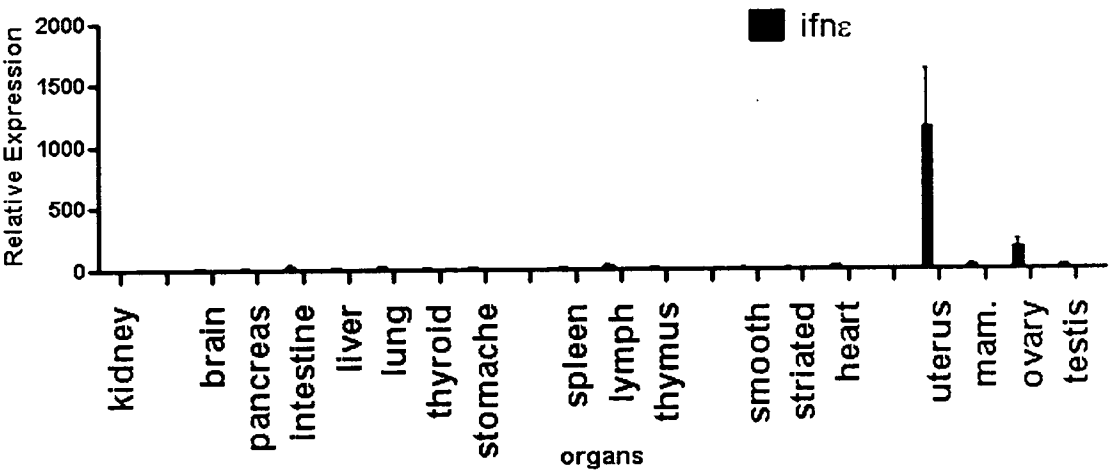


FIGURE 3B

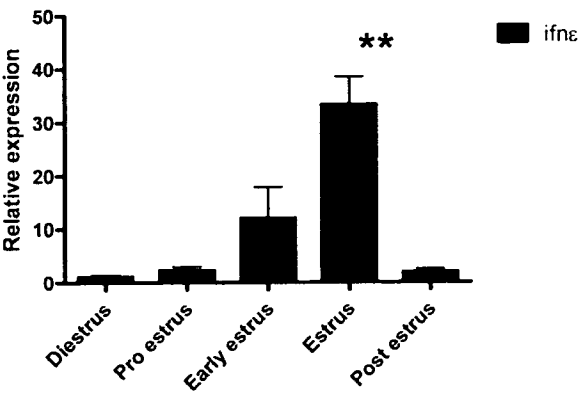
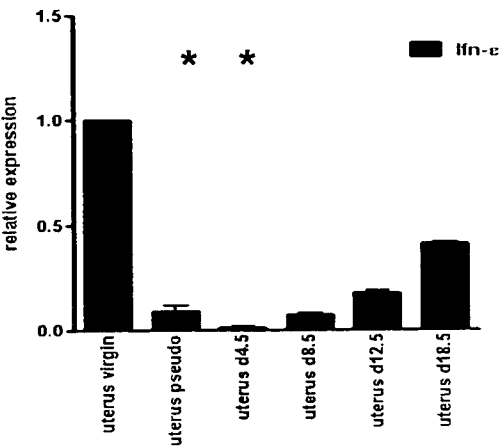


FIGURE 3C



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FIGURE 4A

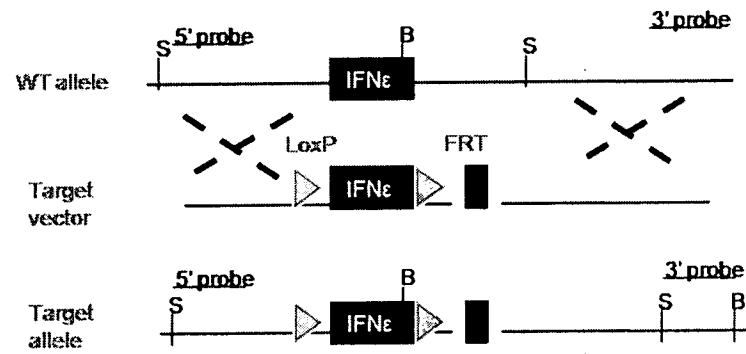


FIGURE 4B

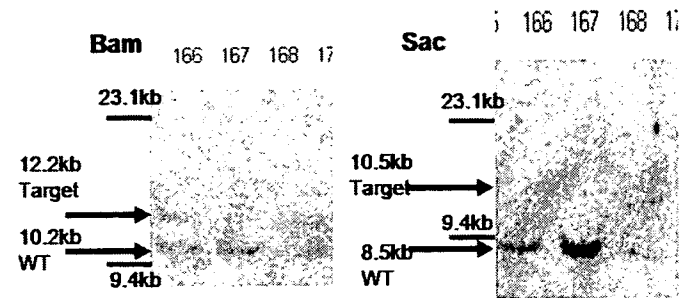


FIGURE 4C

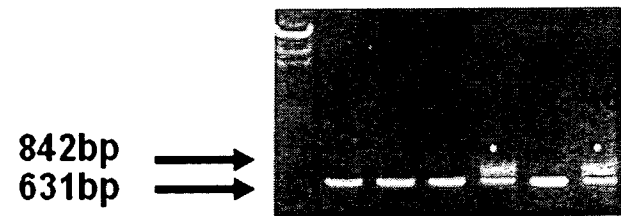


FIGURE 4D

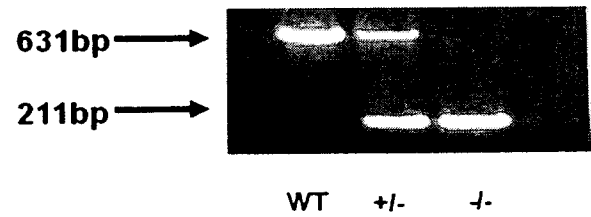


FIGURE 4E

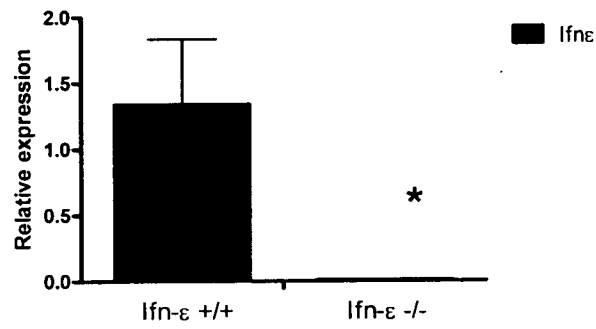
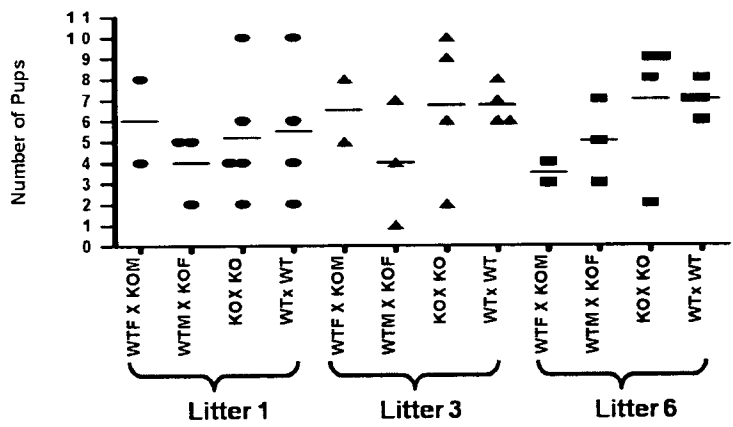


FIGURE 4F



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FIGURE 5A

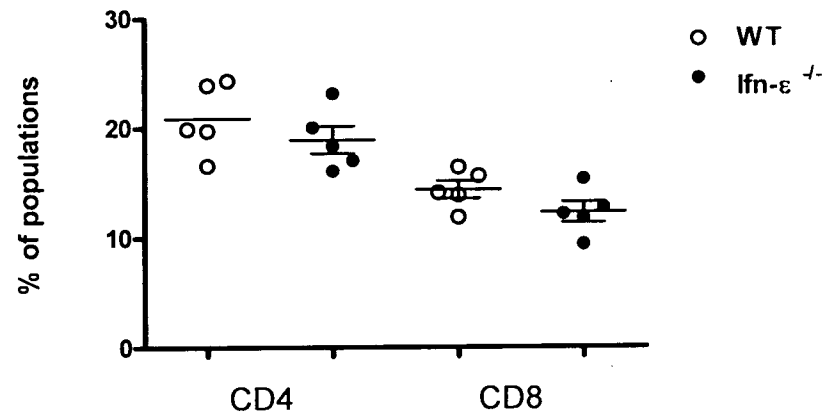
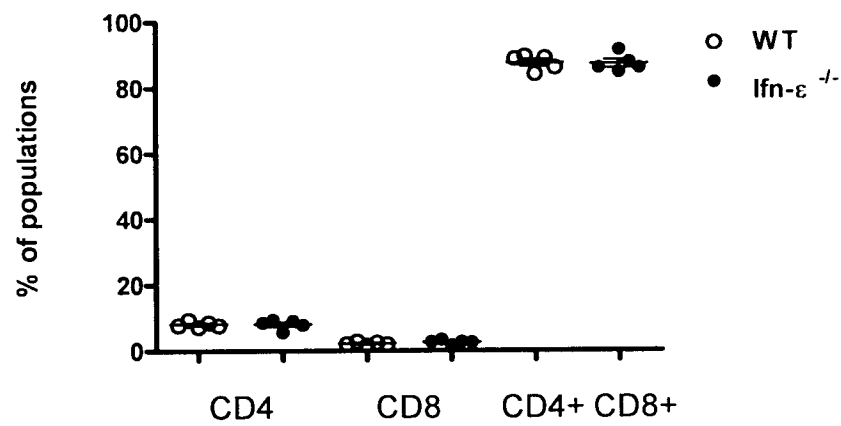


FIGURE 5B



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FIGURE 5C

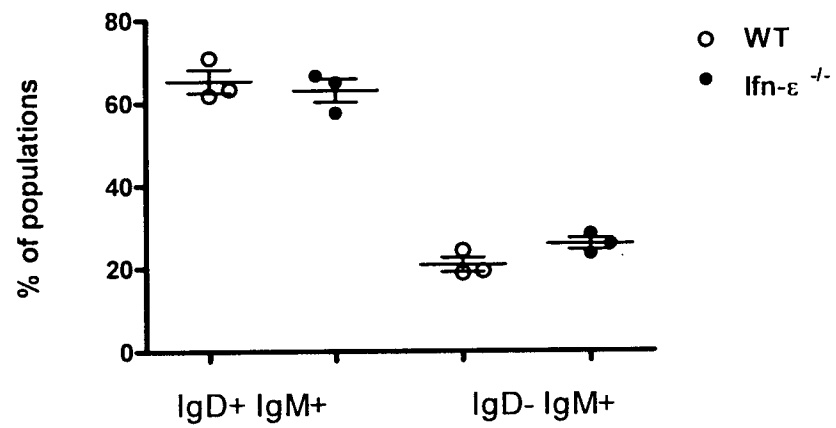


FIGURE 5D

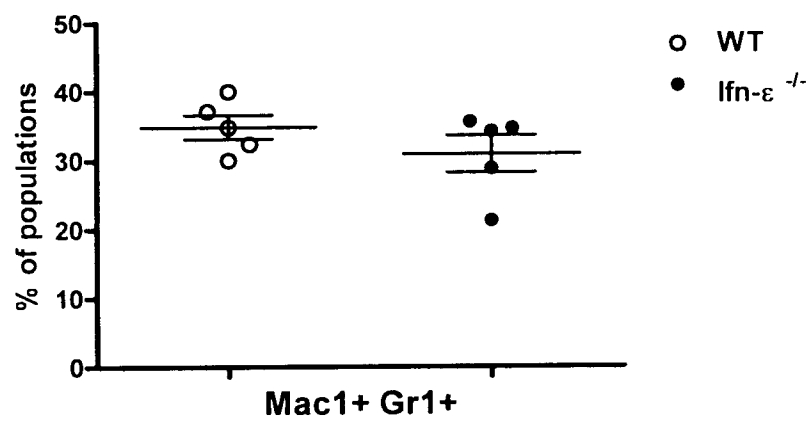
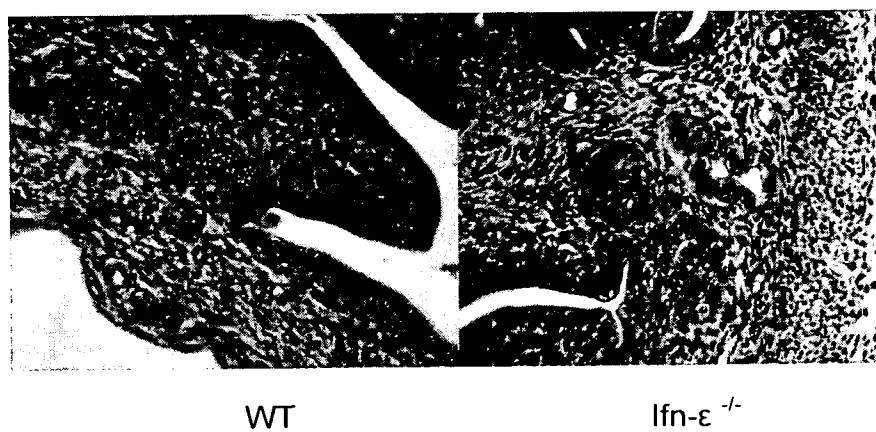


FIGURE 5E



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FIGURE 6A

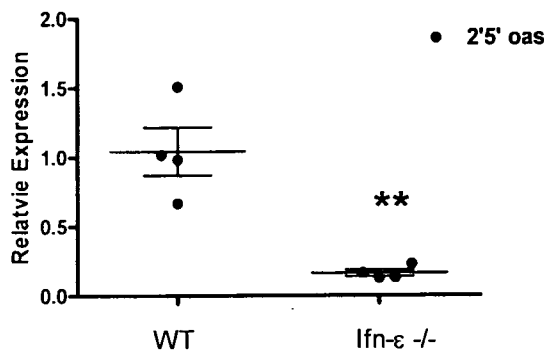


FIGURE 6B

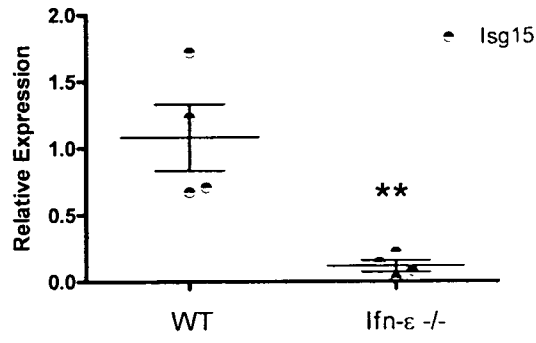


FIGURE 6C

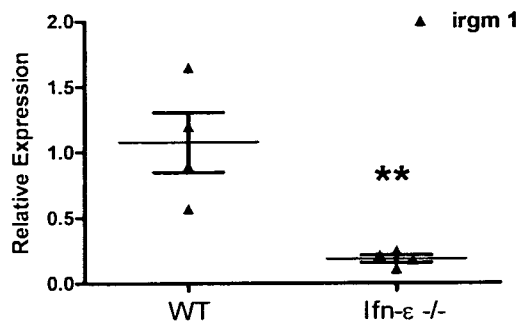
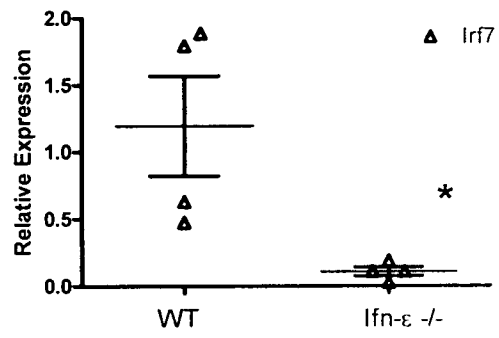


FIGURE 6D



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FIGURE 7A

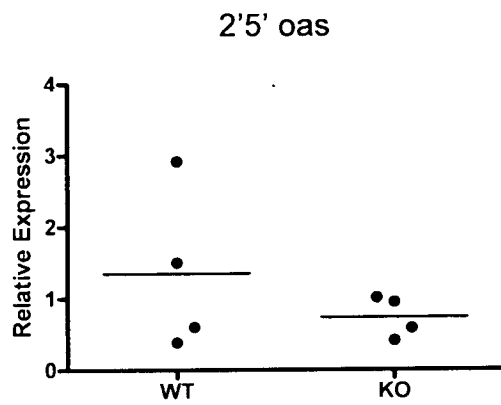


FIGURE 7B

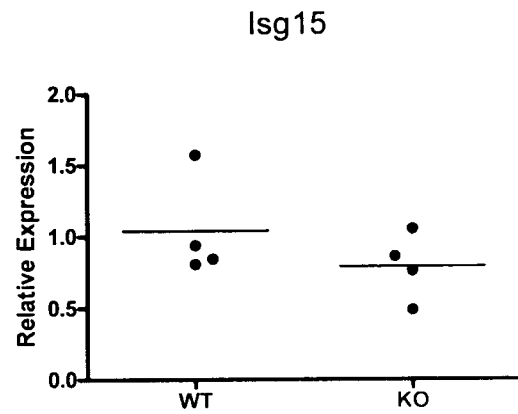
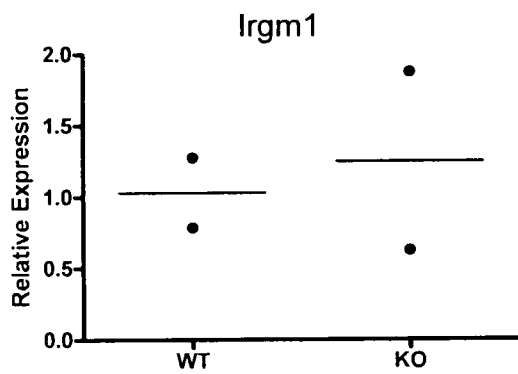
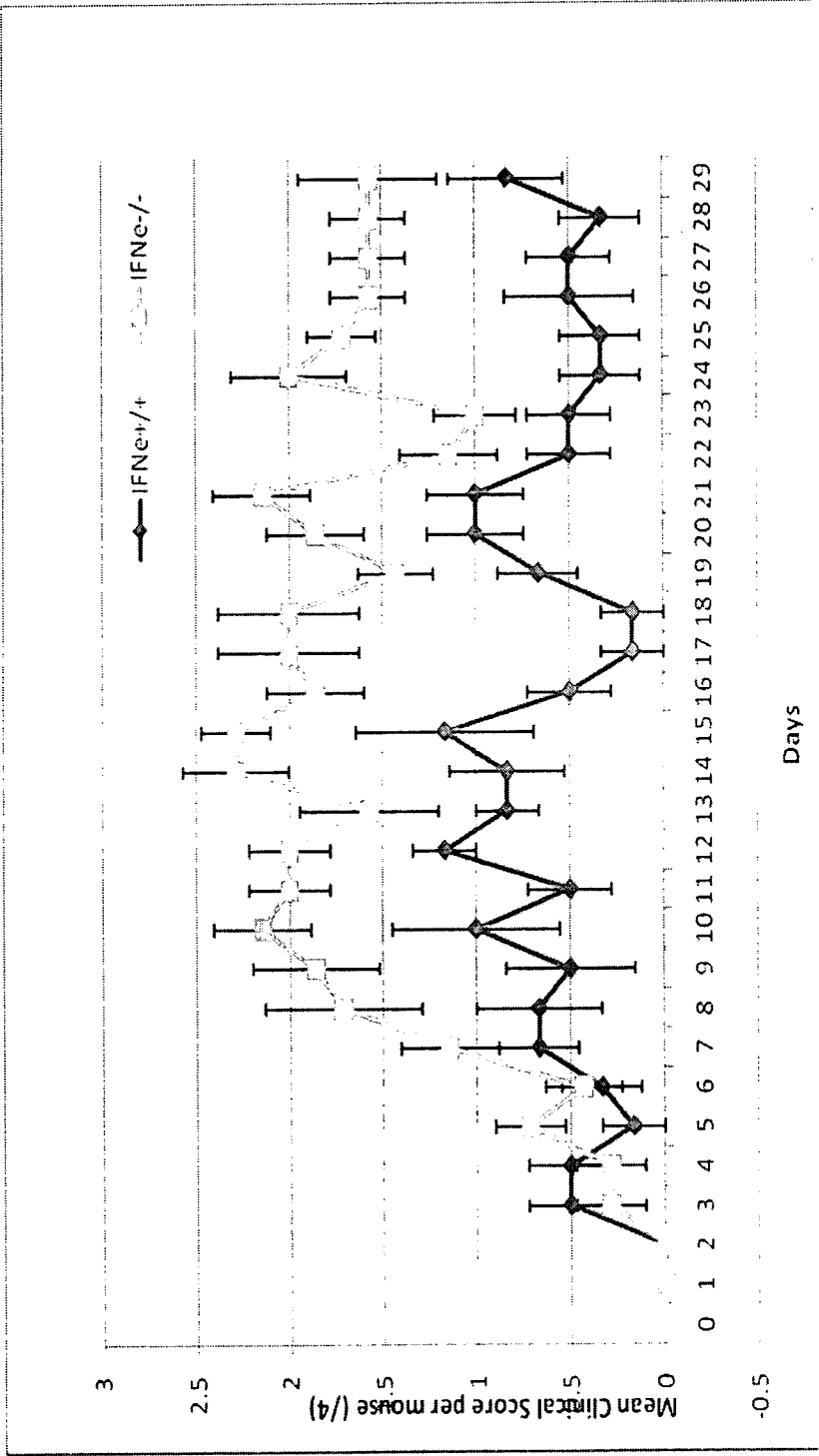


FIGURE 7C



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FIGURE 8A



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FIGURE 8B

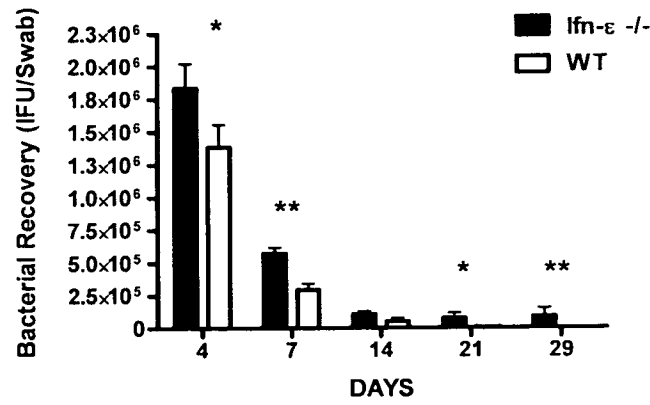


FIGURE 8C

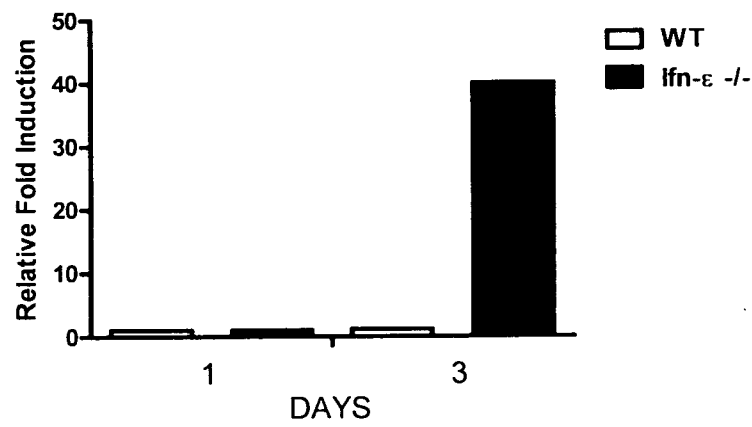
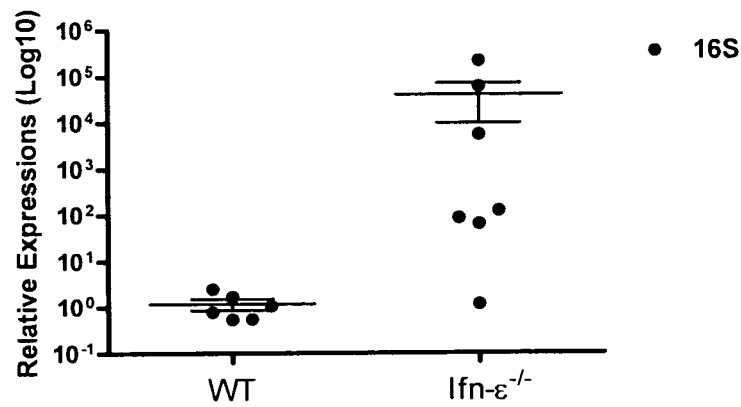
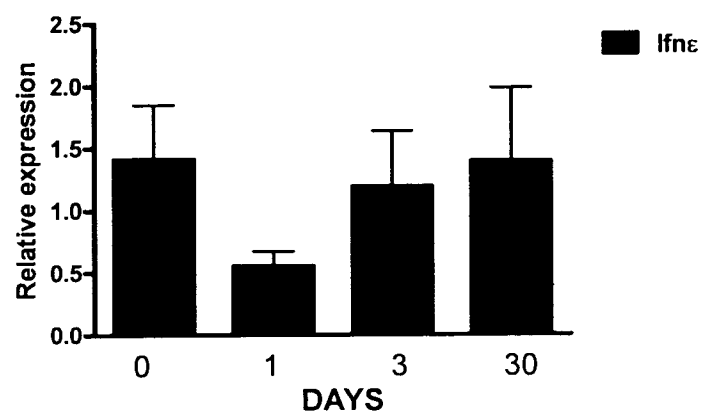


FIGURE 8D



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FIGURE 8E



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FIGURE 9A

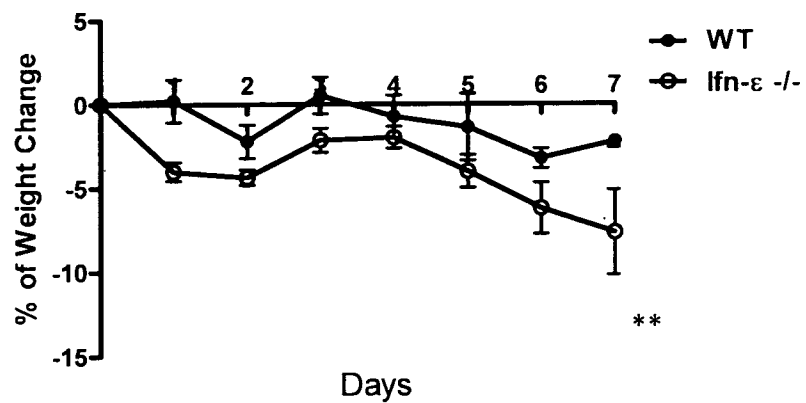


FIGURE 9B

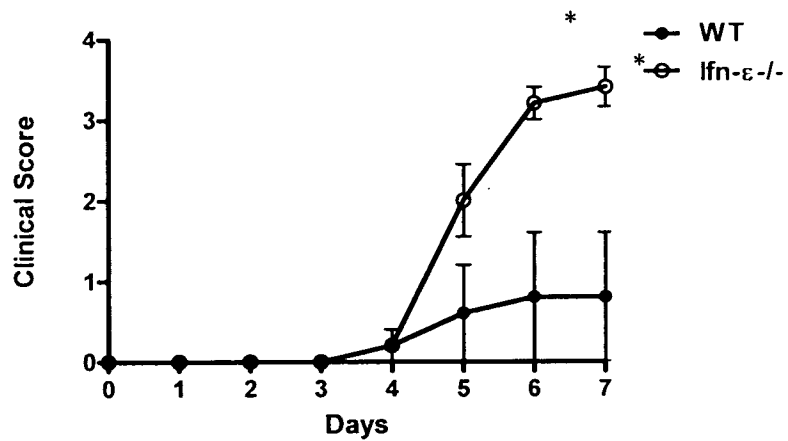


FIGURE 9C

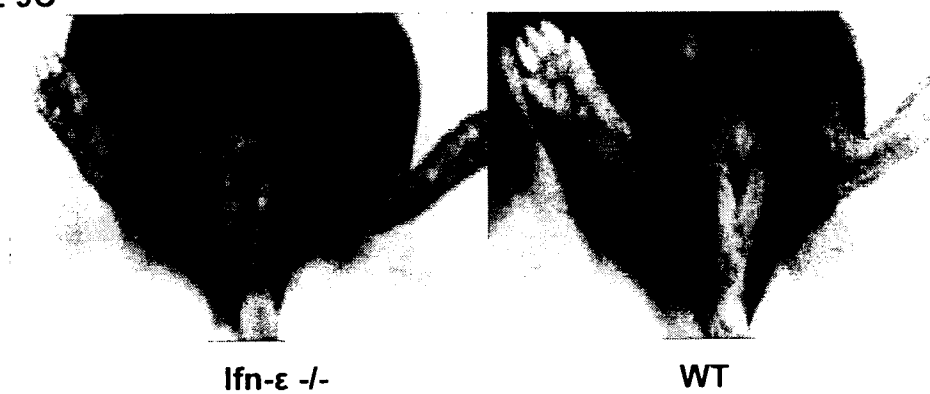


FIGURE 9D

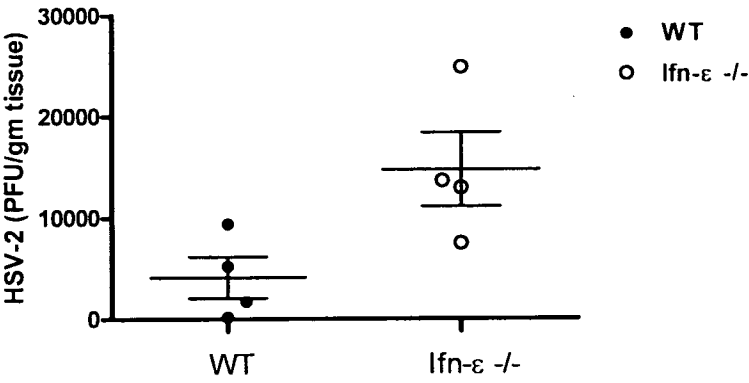


FIGURE 10A

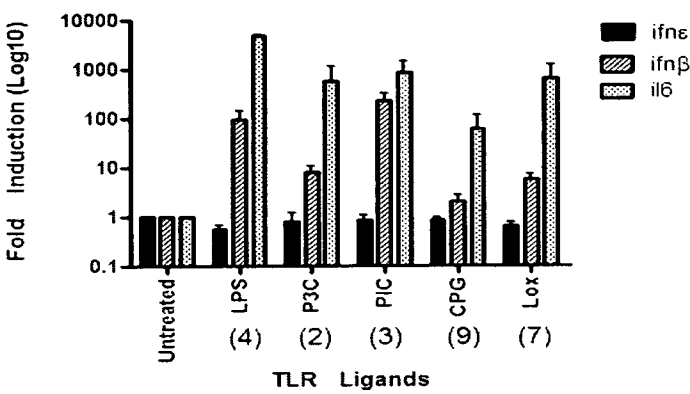


FIGURE 10B

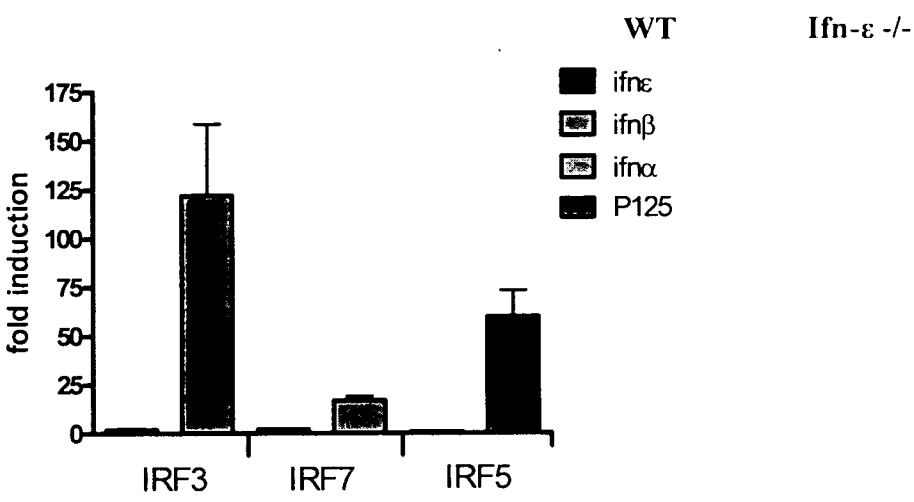


FIGURE 11

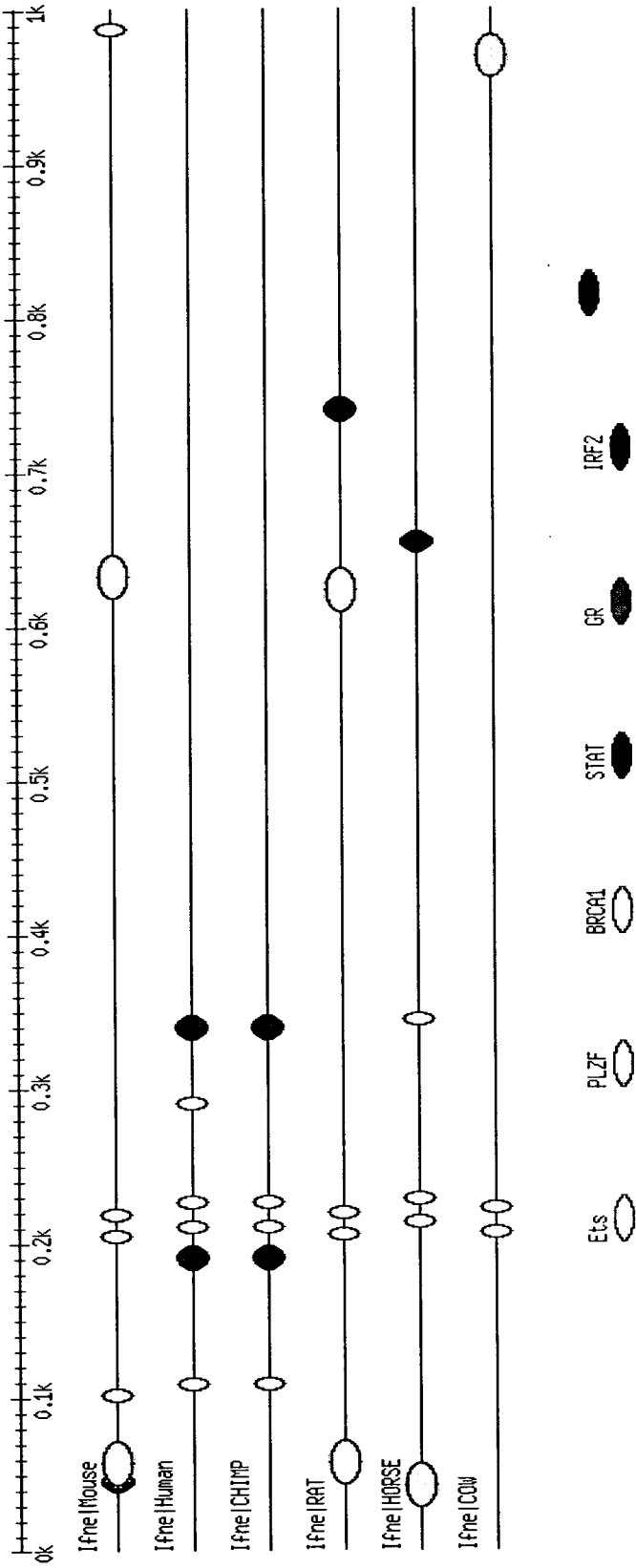
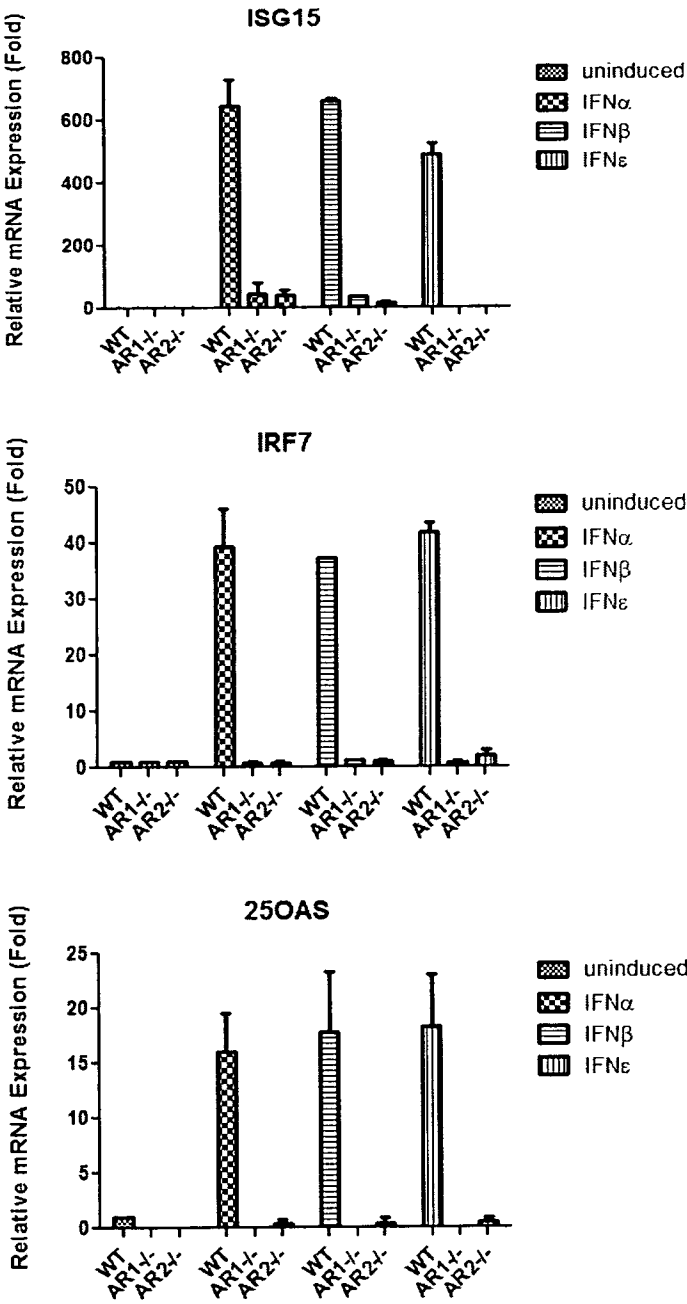


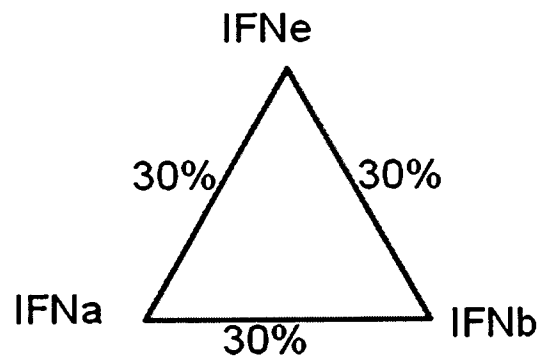
FIGURE 12



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FIGURE 13

A.



B.

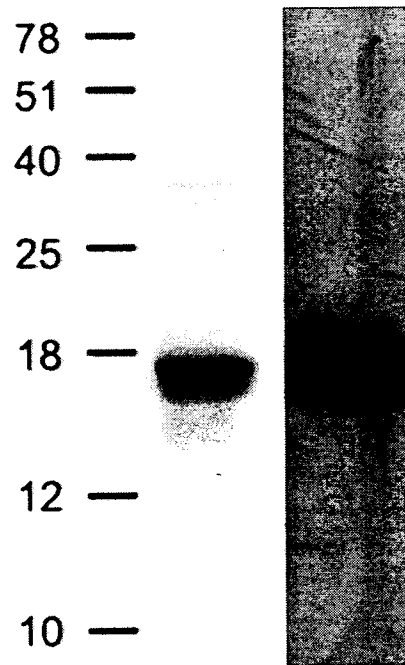
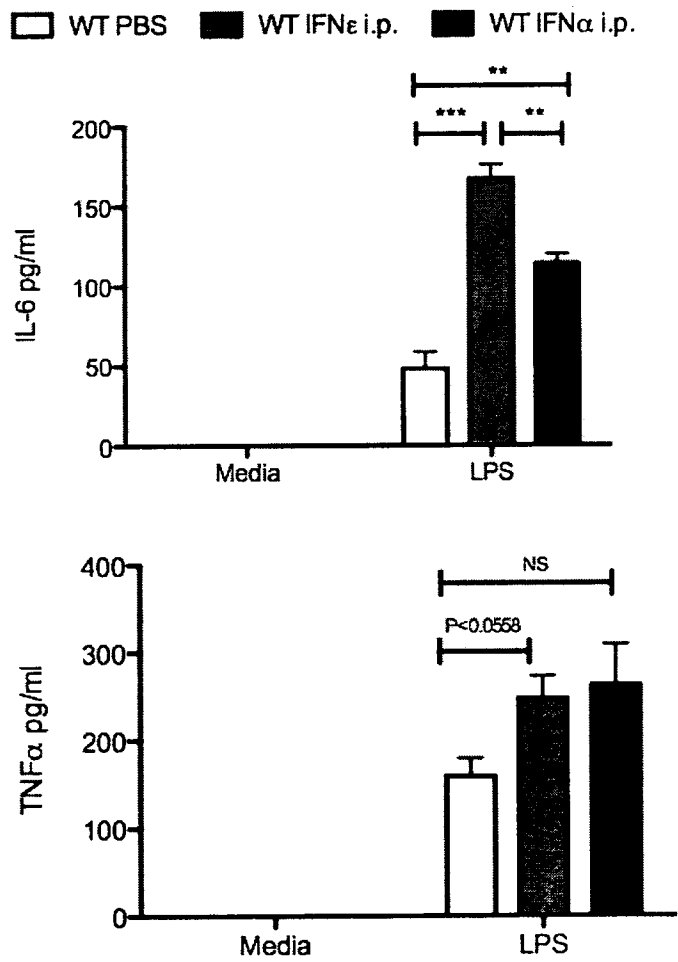


FIGURE 14



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FIGURE 15

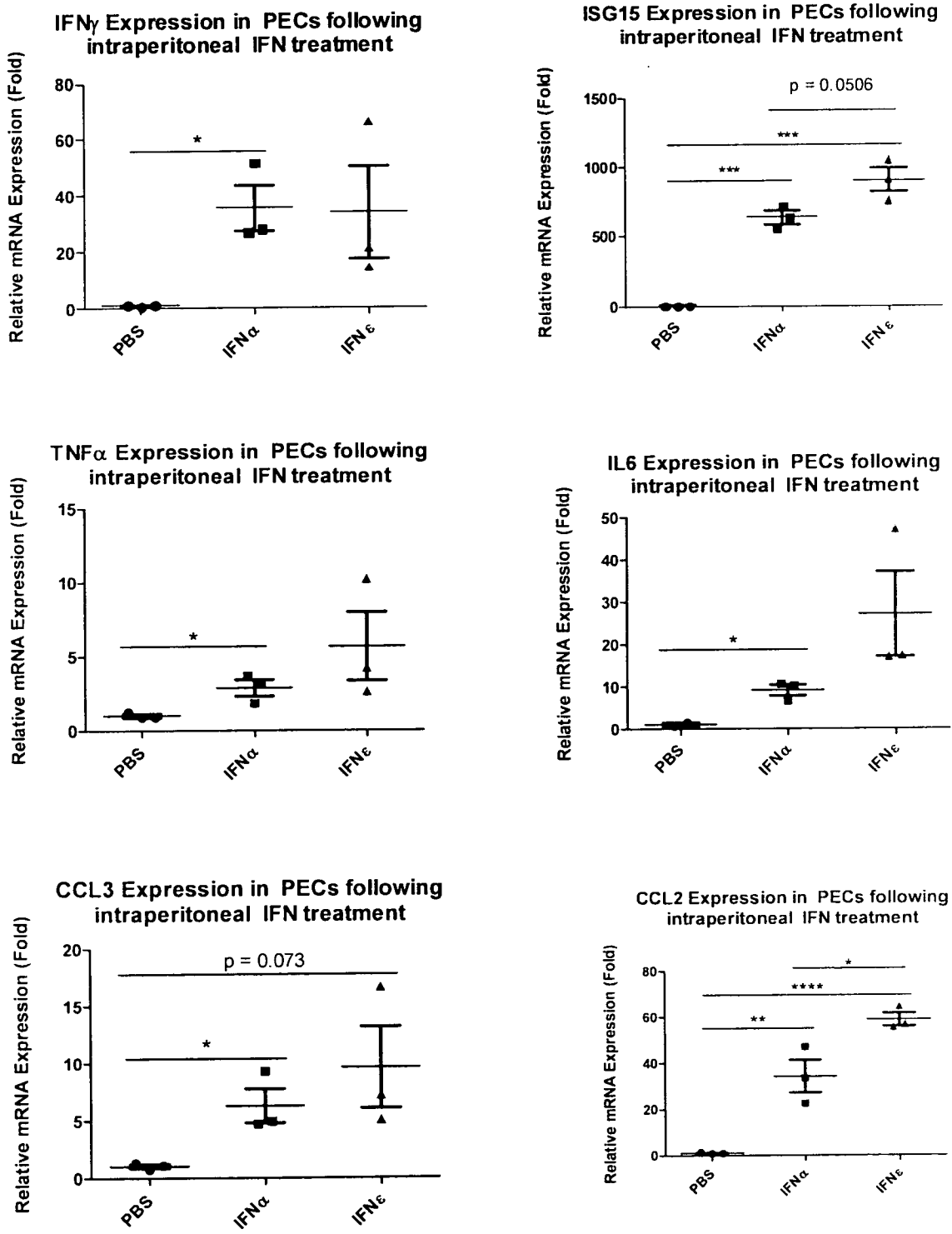


FIGURE 16

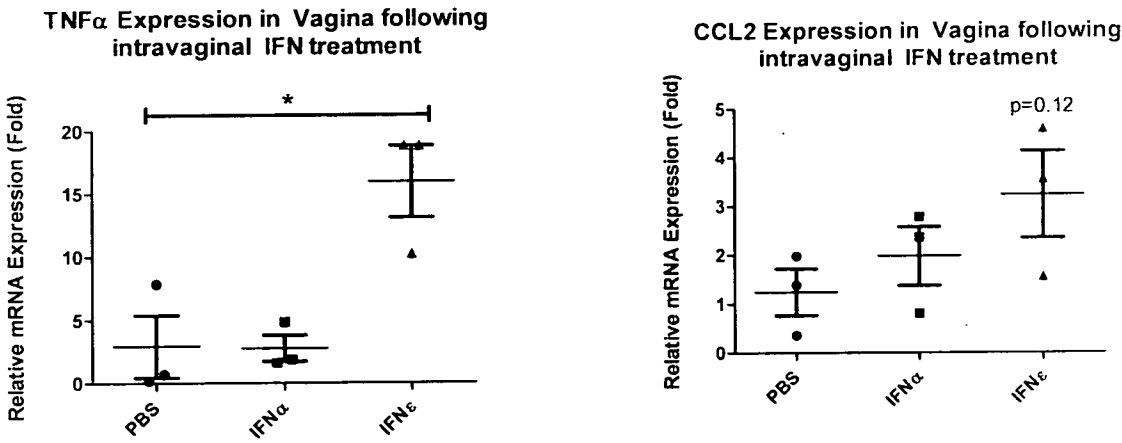


FIGURE 17

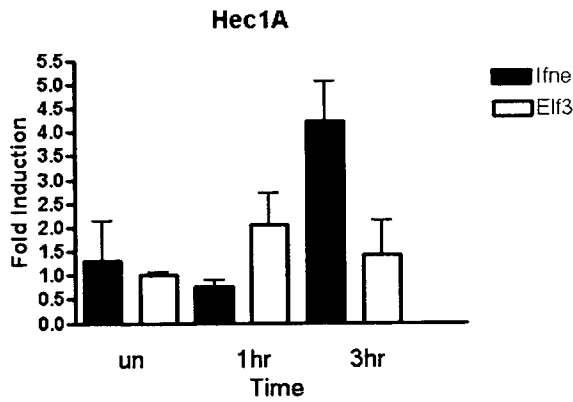
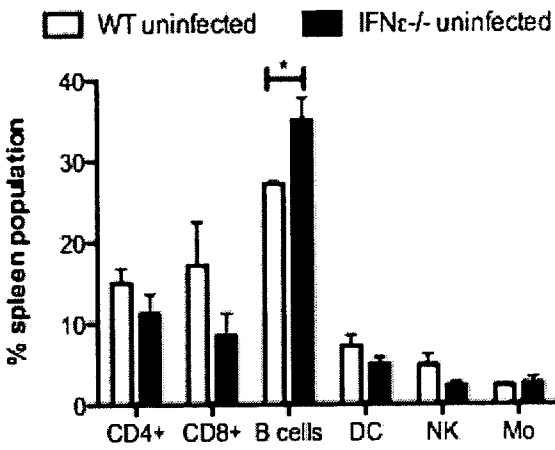


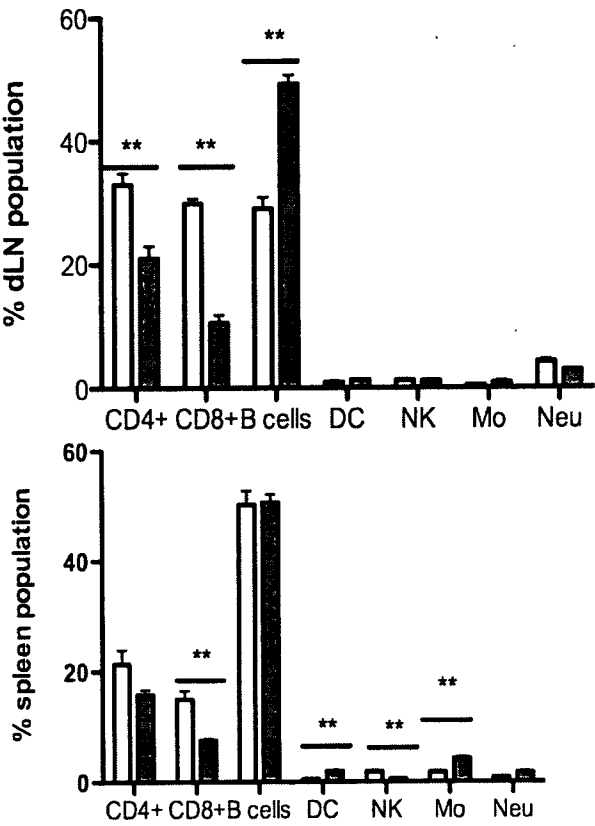
FIGURE 18



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FIGURE 19

□ WT HSV-2-infected ■ IFN ϵ ^{-/-} HSV-2-infected



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2011/000715

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 38/21 (2006.01) *A61K 39/12* (2006.01) *G01N 33/50* (2006.01)
A61K 39/02 (2006.01) *A61K 39/245* (2006.01)
A61K 39/118 (2006.01) *C12Q 1/68* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 EPODOC, WPI, MEDLINE, HCA, BIOSIS (interferon, IFN, e, epsilon, immune, response, system, pathogen, HSV, herpes, HPV, papilloma virus, HIV, immunodeficiency virus, chlamydia, sexually transmitted, STD, STI, reproductive tract, FRT, female, woman, women, ovary, cervix, contraception, abort, estrus, oestrus, menstrual, prevent implantation, terminate, vaccine, adjuvant)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FUNG, K.Y. et al. "Characterisation of a novel, constitutive cytokine that regulates mucosal immunity in the reproductive tract", Cytokine, 2009, Vol. 48, No's 1-2, page 89 (PP1-201). See Abstract.	1 – 8, 15 – 31
X	DAY, S.L. et al. "Differential Effects of the Type I Interferons $\alpha 4$, β , and ϵ on Antiviral and Vaccine Efficacy", The Journal of Immunology, 2008, Vol. 180, No. 11, pages 7158 – 7166. See Abstract, pages 7158 – 7160, 7164, 7165.	9 – 14



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
14 September 2011

Date of mailing of the international search report
20/09/2011

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2011/000715

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2011/000715

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2000/017361 A2 (ZYMOGENETICS, INC.) 30 March 2000. See whole document.	1 – 8, 16 – 31
A	HARDY, M.P. et al. "Characterization of the type I interferon locus and identification of novel genes", Genomics, 2004, Vol. 84, No. 2, pages 331 – 345. See whole document.	
A	MATSUMIYA, T. et al. "IFN- ϵ Mediates TNF- α Induced STAT1 Phosphorylation and Induction of Retinoic Acid-Inducible Gene-1 Human Cervical Cancer Cells", The Journal of Immunology, 2007, Vol. 179, No. 7, pages 4542 – 4549. See whole document.	
A	SHARKEY, D.J. et al. "Seminal plasma differentially regulates inflammatory cytokine gene expression in human cervical and vaginal epithelial cells", Molecular Human Reproduction, 2007, Vol. 13, No. 7, pages 491 – 501. See whole document.	
P,X	MANGAN, N.E. et al. "The role of interferon epsilon in viral infection of the reproductive tract", Cytokine, 2010, Vol. 52, No's 1-2, page 70 (CS5-3). See Abstract.	

Supplemental Box

(To be used when the space in any of Boxes I to IV is not sufficient)

Continuation of Box No: Box III

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1 – 8 (in full) and 16 – 21, 31 (in part). The feature of treating a condition characterised by a pathogen infection in a female mammal comprising the administration of an agent which upregulates IFN ϵ , is specific to this group of claims.
- Claims 9 – 14 (in full) and 16 – 21, 31 (in part). The feature of inducing an immune response directed to a pathogen, comprising the administration of a pathogen derived antigen and an agent which upregulates IFN ϵ , is specific to this group of claims.
- Claims 15 (in full) and 16 – 21, 31 (in part). The feature of preventing implantation of a zygote into the endometrium of a mammal or effecting abortion, comprising administration of an agent which upregulates IFN ϵ , is specific to this group of claims.
- Claims 22 – 30 (in full) and 31 (in part). The feature of screening a female mammal for a predisposition to developing a pathogen infection, comprising the measurement of IFN ϵ expression levels, is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is the administration/upregulation/measurement of IFN ϵ . However this feature does not make a contribution over the prior art because it is disclosed in the prior art. For example:

- FUNG, K.Y. et al. "Characterisation of a novel, constitutive cytokine that regulates mucosal immunity in the reproductive tract", Cytokine, 2009, Vol. 48, No's 1-2, page 89 (PP1-201)
- DAY, S.L. et al. "Differential Effects of the Type I Interferons α 4, β , and ϵ of Antiviral and Vaccine Efficacy", The Journal of Immunology, 2008, Vol. 180, No. 11, pages 7158 – 7166

The prior art documents disclose the expression of IFN ϵ in the female reproductive tract and the role of IFN ϵ in muscosal immune responses.

In the light of these documents this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2011/000715

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	2000/017361	AU	60425/99	CA	2342773
		JP	2002526078	EP	1141296
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.					
END OF ANNEX					