(54) Title: VIRUS-RESISTANT TRANSGENIC ANIMALS

(57) Abstract

A transgenic animal with enhanced viral resistance transmissible to its offspring is prepared by introduction of a gene encoding a heterologous interferon having antiviral activity into a host animal. The gene encodes the interferon of a different animal species and therefore its expression product is less toxic to the host at an embryonic, fetal, neonatal or juvenile stage of development. Preferably, the human beta interferon gene is introduced into the cells of a non-human animal.
FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

VIRUS-RESISTANT TRANSGENIC ANIMALS

BACKGROUND OF THE INVENTION

5 Field of the Invention

This invention relates to the production of virus-resistant transgenic animals.

10 Information Disclosure Statement

Viral infections in agricultural animals represent a major economic loss for farmers and ranchers. Significant viral diseases in cattle are infectious bovine rhinotracheitis, parainfluenza, bovine viral diarrhea, rabies, and foot and mouth disease. In swine, transmissible gastroenteritis, swine mycoplasmal pneumonia, pseudorabies, and rabies infections are serious health problems. Poultry production is hampered by Marek's disease, Newcastle disease, infectious bursal disease, and infectious bronchitis, all of which are viral diseases. In 1981, over $500,000,000 was spent to treat agricultural animals against viral infections and the economic loss to agriculture resulting from viral disease is substantially greater than this cost.

One method of providing virus resistance is by use of vaccines. Vaccination may be with attenuated or inactivated virus, or with viral proteins produced by genetic engineering techniques. Vaccination, unfortunately, has many disadvantages. The animals must receive separate immunizations for each disease-causing organism. Moreover, the immunity diminishes with time and must be restored by follow-up
immunizations, a practice which is inconvenient in the case of livestock allowed to roam on free range.

Another approach is that of treatment with an antiviral drug. Such drugs include acyclovir, ribovirin, adenine arabinoside and amantadine. A drug of particular interest is interferon, or, more especially, the various species of alpha (leukocyte) and beta (fibroblast) interferon. Collectively, these are called Type I interferons. The antiviral activity of various mammalian interferons is reviewed in Colowick, S. P. and Kaplan, N. O. Interferons, S. Pestka (Ed.), Methods in Enzymology. Orlando: Academic Press, Inc., 1986, Vol. 119(C). The interferons do not act directly against the virus, rather, they stimulate the antiviral response of the immune system. The antiviral activities of exogenous interferons in animals are reviewed by Stewart II, THE INTERFERON SYSTEM, 282-283, Table 16 (2d ed. 1979).

Unfortunately, the antiviral drugs are expensive and provide protection for only a short period of time.

Kawade, et al., at the 1986 ISIR-TNO Annual Meeting, September 7-12, 1986, reported the production of transgenic mice carrying mouse interferon beta or gamma genes coupled to the mouse MT-I or heat shock promoters. According to Kawade, linearized plasmid DNA was microinjected into fertilized mouse eggs. Twenty-eight pups were born, four of whom carried the chimeric IFN genes. Three of these transmitted the genes to offspring. Apparently, Kawade had not determined whether the chimeric gene was actually expressed in the mice, but had already been able to express it in cultured cells induced by Ca^{2+} or by heat. Our invention relates to the introduction of the interferon
gene of another animal species into a host animal, and to the production of a healthy transgenic animal with enhanced viral resistance which it can genetically transmit to its offspring.

Gordon and Ruddle, Science, 214:1244 (1981) claimed integration and stable germline transmission of genes injected into mouse pronuclei. Ten thousand copies of a human leukocyte IFN gene were microinjected into each zygote. Ten mice were born from 33 implanted embryos; one of the mice was transgenic. Expression was not reported, and the purpose of the study was to show that the technique could be used to study gene action during mammalian development.

Staehelin, WO 87/00864 (publ. February 12, 1987) observed inhibition of influenza virus in NIH 3T3 mouse cells genetically modified to express the mouse protein Mx. This protein is one whose production is stimulated by mouse interferon. Staehelin suggested that an animal could be protected against viral infection by inserting a gene encoding the Mx protein into that animal, preferably at the single cell embryo stage. He taught that the gene of choice would be the one encoding the Mx protein that is normally found in the species of animal to be protected. He did not suggest use of the interferon gene, or of a gene derived from a different species than the host.

The expression of an exogenous gene in a transgenic animal is a far more subtle process than the expression of the same gene in mammalian cell culture. Expression of the gene at the wrong time may kill the animal. The problem is particularly acute when the gene is inserted into an embryonic cell, since the
developing embryo, fetus or neonate may be distinctly sensitive to the gene product. It is known that mouse interferon is toxic to newborn mice. Gresser, et al., Nature, 258: 76-78 (1975).

There is considerable controversy as to the cross-species antiviral activity of interferon.


and suggests that it may be useful in treating viral infection (’993, claim 36). No reference is made to the creation of transgenic animals.


Mark, US 4,588,585 relates to an IFN-beta analogue. It is suggested that this protein may be useful in diagnosis and treatment of viral and other infections. (col. 20)


Thompson, EP Appl 217,645 describes a stabilized IFN-beta or IL-2 composition for antiviral use.

TORAY, JP 61052285 (abstract) discloses expression of IFN-beta in a eukaryotic cell, under control of, for example, the MT promoter.


Sugano, WO 82/02715 (abstract) describes expression of IFN-beta in a eukaryotic cell.

Palmiter, WO83/01783 teaches that a structural gene may be coupled to the MT promoter, introduced into the embryo of a mouse, and expressed in the adult mouse
after induction with heavy metals. Palmiter worked with both thymidine kinase and rat growth hormone. Palmiter suggested that the MT promoter could be used to control transcription of other genes in higher animals, including genes relating in some manner to "disease resistance". (page 31).

Hamer, WO84/02534 discloses expression of HGH in mammalian cell culture under control of the MT promoter.

Karin, US 4,601,978 generally suggests inducible expression of heterologous genes in mammalian cells under the control of the human MT II gene.

Kushner, WO86/04920 relates to expression of HGH and certain other proteins (IFN is not mentioned) under the control of the human MT II promoter in CHO cells.

The foregoing information is disclosed because it might be considered material by the Examiner in the course of examining this application. No admission is made that any of this information constitutes prior art or pertinent prior art, that the references themselves accurately or completely describe the work reported, or that the dates of publication given are exact.

SUMMARY OF THE INVENTION

This invention rests in the production of transgenic animals with inherent viral resistance which will persist throughout their lives and, in a preferred embodiment, be transmitted to their offspring. This viral resistance will be conferred by the gene encoding an alpha or beta interferon, preferably the latter, of
an animal species other than that to which the host animal belongs. Preferably, the human interferon gene is introduced into the genome of animals other than man.

The native interferon gene is expressed, if at all, only at very low levels, prior to its induction by virus. However, it is desirable to provide for interferon expression prior to the onset of viral infection. In one embodiment of this invention, a promoter is used which is at least partly constitutive, so that significant amounts of interferon are expressed by the animal at all times.

It may be undesirable, however, to use an interferon gene which is native to the animal species to which the host animal belongs (a "homologous" gene). The expression production of that gene is likely to be more active in the host than that of a heterologous gene, and therefore more likely to have an adverse effect. Since human beta interferon is less active in mice, for example, than in our own species, its continuous delivery is less likely to have a toxic effect. Since the human interferon is expressed intracellularly, the presence or absence of cell surface receptors for human interferons is irrelevant.

In another embodiment, the promoter is a wholly or partly inducible promoter which is induced prior to the onset of viral infection, and, preferably, when the risk of infection is increased. For example, expression might be induced prior to shipping, or when a neighbor's herd is infected.

The claims appended hereto are incorporated by
reference as descriptions of the preferred embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the construction of pMTIF-beta1A from pMK and pIFR-beta-IFN.

Figure 2 is a southern blot of restriction fragments (double digestion with EcoR1 and PvuII) of DNA from the tails of transgenic mice. Each lane received 10 ug DNA, which was electrophoresed on a 0.8% agarose gel at 50v for 15 hrs., then transferred to a nitrocellulose filter. The 3.1kb spots correspond to the EcoR1-PvuII fragment of the human IFN beta gene.

Figure 3 shows an antibody-antigen neutralization CPE assay. Rows and A and B contain serum from transgenic mice. Row B additionally contains anti-human IFN beta antibody. Rows C-F all contain serum from normal mice. C also contains IFN standard; D, IFN standard plus antibody, and F, antibody. Row G contains IFN standard only. Row H contains cell (M1 to M6) or virus (H-7 to H-12) controls. The columns represent successive two-fold dilutions of the above from the contents of the first column. All samples were incubated overnight with human WISH cells, challenged with VSV for 48 hours, and stained with crystal violet. It will be noted that the serum from the transgenic mice apparently contains human beta interferon, as it protects human cells from viral infection and is neutralized by an anti-human beta interferon antibody.

Figure 4 shows the antiviral effect of human beta interferon in transgenic mice challenged with pseudorabies virus at three different levels of
In panels A and B, it is seen that the "A gene" appears
to impart some resistance to the virus, which is more
apparent in panel B. In panel C, we see the relative
effects of the A and B gene.

DETAILED DESCRIPTION OF THE INVENTION

The term "animal" is used herein to include all
vertebrate animals, except humans. It also includes an
individual animal in all stages of development,
including embryonic and fetal stages. A transgenic
animal" is any animal containing one or more cells
bearing genetic information received, directly or
indirectly, by deliberate genetic manipulation at a
subcellular level, such as by microinjection or
infection with recombinant virus. The term is not
intended to encompass classical cross-breeding or in
vitro fertilization, but rather is meant to encompass
animals in which one or more cells receive a
recombinant DNA molecule. This molecule may be
integrated within a chromosome, or it may be
extrachromosomally replicating DNA. The term "germ
cell line transgenic animal" refers to a transgenic
animal in which the genetic information was introduced
into a germ line cell, thereby conferring the ability
to transfer the information to offspring. If such
offspring in fact possess some or all of that
information then they, too, are transgenic animals.

The information may be foreign to the species of
animal to which the recipient belongs, foreign only to
the particular individual recipient, or genetic
information already possessed by the recipient. In the
last case, the introduced gene may be differently
expressed than the native gene.

The transgenic animals of this invention may be of any vertebrate species, other than man, which is responsive to interferon. Farm (e.g., horse, cow, pig, goat, sheep) and pet (e.g., dog, cat) animals are of particular interest. Interferons have been demonstrated in fish, reptiles, birds, and mammals, including primates, felines, bovines, canines, and rodents. The interferon gene may likewise be derived from any vertebrate species other than the species of the intended host animal. The human, bovine and murine interferon genes are the best characterized. See Weissmann and Weber, Progr. Nucl. Acids Res. & Mol. Biol., 33: 251-300 (1986). The use of human interferon gene is preferred.

The interferon gene may be any interferon gene species which cells express in response to viral infection. These presently fall into two major classes, alpha and beta interferon, which in turn may be divided into various subclasses. However, this invention would encompass use of other classes of interferon having antiviral activity. Besides using the naturally occurring gene, one may use genes which have been modified for altered expression in the intended host or for production of a modified protein having enhanced antiviral activity.

The introduced interferon gene generally should not encode the same interferon as the interferon native to the host animal, and its expression product preferably should be more homologous to the interferon of a different animal species than to that of the host animal. However, it is conceivable that a more
modestly modified interferon will fall within the compass of the present invention if, it is of reduced toxicity. It is also conceivable that a minor interferon species, native to the host, will have sufficiently low activity to be useful in the present invention.

The genes may be obtained by isolating them from genomic sources, by preparation of cDNAs from isolated mRNA templates, by direct synthesis, or by some combination thereof. The published sequences of numerous alpha and beta IFN genes greatly facilitates obtaining a gene encoding an antiviral IFN, and this invention is not limited to the use of any particular gene. However, the human beta-1 interferon gene is preferred. It is important that the added gene have sufficient activity to enhance viral resistance but not enough to induce other, toxic responses.

To be expressed, the structural gene must be coupled to a promoter in a functional manner. If a constitutive promoter is used, a viral promoter, such as the SV40 early promoter, is preferred. The MT promoter, while often referred to as an "inducible" promoter, is best described as "semi-constitutive," since it is "on" all of the time, even though its activity is boosted by heavy metal ions. Such promoters, as well as pure inducible promoters, may also be used. Promoter/regulatory sequences may be used to increase, decrease, regulate or designate to certain tissues or to certain stages of development the expression of interferon. The promoter need not be a naturally occurring promoter.

In mammals, the exogenous interferon gene may be
introduced by zygote microinjection as described below for the mouse. In avian species, the interferon gene may be introduced through the use of avian retroviral vectors. The interferon gene may also be introduced into non-germline cells (somatic cells) in a fetal, newborn, juvenile or adult animal by retroviral vector-mediated genetic transformation of somatic cells of one or more tissues within the animal.

Example 1: Construction of MT-IFN Plasmid


The sequence is incorporated by reference herein. An EcoRI/Bgl II 1.8 kb MT-I promoter was fused to the h-IFN-beta structural gene by the following procedure: pMK was linearized by Bgl II and pIFR was linearized, by Nco I; then both plasmids were blunt ended by a Klenow "filling-in" reaction. Both blunt ended plasmids were subsequently cut by EcoRI which left both plasmids with two compatible ends: EcoRI and blunt. Then 1.8 kb MT-I promoter was inserted into pIFR by a sticky/blunt ligation. This plasmid was named pMTIF-beta A. Because of the DNA sequence generated at the
junction of the MT-I promoter sequence and the interferon structural gene, an ATG codon was introduced into the resulting fusion gene. The nucleotide sequence ATG codes for the initiation of transcription of the DNA sequence into RNA, but initiation from this "spurious" ATG results in an RNA message in an improper reading frame which is transcribed into a protein other than interferon. The appropriate ATG sequence in the correct reading frame to express authentic human interferon is also present within this fusion gene. Competition between the two ATG initiation sites results in a decrease in the amount of authentic human interferon message. Therefore, the additional ATG is eliminated by the following method: pMTIF-beta was first linearized by NcoI cleavage, followed by a nuclease S1 "chewing back" reaction (because additional ATG codon is right on the 5' overhang, single strand nuclease activity of nuclease S1 will blunt end the Nco I site so that the additional ATG will be deleted). Finally, the plasmid was recircularized by blunt-end ligation. This plasmid was named pMTIF-beta B.

Example 2: Microinjection of B-IFN Gene Into Mouse Zygotes and the Birth of Mice Containing the B-IFN Gene In Their Chromosome

Linear DNA sequences (approx. 500 copies of a 3.1 kb EcoRl/Hind III fragment) from pMTIF-beta [A] and [B] were microinjected separately into the male pronucleus of fertilized mouse eggs. The eggs were transplanted into pseudopregnant female mice for gestation to term. At 3 to 4 weeks of age tissue from the tail of these mice was removed. Their DNA was analyzed for the presence of the integrated pMTIF-beta sequences within the mouse chromosomal DNA, as shown by hybridization to
radioactively labeled pMTIF-beta probe DNA. Both "dot blot" and "Southern blot" hybridizations were performed (Molecular Cloning: A Laboratory Manual, T. Maniatis, E. F. Fritsch and J. Sambrook). For Southern blot hybridization, the chromosomal DNA was digested into fragments using restriction endonuclease which will cut the pMTIF-beta sequences into known lengths, both enzymes which cut twice within the pMTIF-beta sequences to demonstrate the authenticity of the added gene and once within the pMTIF-beta sequences and again within the adjoining chromosomal sequences to demonstrate integration. The 3.1 kb fragments which hybridize to probe DNA show the authenticity of the MTIF-beta sequences and the larger "junction" fragments demonstrate that the MTIF-beta sequences are integrated into the mouse chromosomes (see Figure 1). Quantitation of the dot blot and Southern blot hybridizations indicate that mice having from 2 to 400 integrated copies of MTINF-beta were produced. Four transgenic mice with the pMTIF-beta [A] sequences in their chromosomal DNA out of 140 mice produced were found. Two transgenic pMTIF-beta [B] mice were produced out of 40 animals produced. These animals were mated and offspring produced. For all except one animal the integrated gene was transferred in a Mendelian fashion. One mosaic animal was identified which produce offspring which showed Mendelian genetics after the third generation.

Example 3: Expression of Authentic Human Interferon Beta in Transgenic Mice Containing Integrated MTIF-beta A or B Sequence.

In order to demonstrate the capability of the MTIF-beta A and B fusion genes to express authentic
human interferon in the mouse, secretions from mouse cells transfected with these sequences and serum from transgenic mice containing these sequences in their chromosomal composition were assayed for specific protection provided to human cells against viral infection using established standard human interferon assays.

Both pMTIF-betaA and pMTIF-betaB were used for a transient expression assay. The cell used was mouse L cell (in which MT-I promoter worked better than other cell lines considered, such as mouse 3T3 cells). When L cells are about 80% confluent, they are washed twice in DMEM minus serum (to get rid of nuclease activity), and 1.0 ug/ml of DNA (pMTIF-betaA OR betaB) and 0.2 mg/ml DEASE-dextran in DEAE minus medium are added to about 500,000 cells/ml. The mixture is incubated for one hour, then the DNA solution is removed and 10% DMSO in HEPES buffered saline is added to the cells for 90 seconds at room temperature. Next, the cells are washed first with PBS and then with growth medium (DMEM supplemented with 10% calf serum and gentamicin). Finally, growth medium is added to the cells and the cells are incubated at 37°C for five days. Medium is changed every 24 hours up to the fifth day. Then the medium is used for cytopathic effect (CPE) assay and plaque reduction assay (PR). Familletti, et al., Methods. Enzymol., 78: 387-94 (1981). The principle of the two assays are the same: if there is human interferon in the media, the human cells incubated with the medium should be resistant to viral infection. The differences between the two assays are that in the plaque reduction assay, the exact number of plaques formed by viral infection can be counted but much more sample has to be used. For CPE assay, 96-well
microtiter plate is used so that much smaller sample can be applied and serial dilutions can be easily carried out. However, in CPE assay, the number of the plaques cannot be counted and 50% killing (or protection) is arbitrarily assigned (very well trained eyes are needed for making this assignment). Thus, the viral plaque reduction assay was used for quantification and the CPE assay was used for both qualitative and quantitative purposes.

For performing the plaque reduction assay, small petri dish plates are used (with grids). First, human WISH cells are grown to confluency on the plates, then the media are removed, and plates are washed with PBS. The cells are subsequently incubated with media collected from the transient expression assay at different dilutions for at least 6 hours (usually it is overnight). Then the media is removed again, the cells are washed with PBS and then incubated with 50 PFU of vesicular stomatitis virus (VSV) for 30 minutes at 37°C followed by pouring methycellulose-medium (serves as soft agar) on top of the cells. Next, the plates are incubated at 37°C for 48 hours. Then the incubated plates are stained with methylene blue and the plaques are counted.

The major conclusion which were drawn from these PR assays were:

a. media collected from transiently transfected cell culture showed anti-viral activities. They could protect human WISH cells from VSV infection whereas non-transfected cell media could not; more significantly, the media from pMTIF-betaB transfected cell cultures showed higher antiviral activity than
that from pMITF-betaA transfected cell cultures, which indicated that mRNA transcribed from pMTIF-betaB is a more efficient transcript than mRNA from pMTIF-betaA. In other words, mRNA of pMTIF-betaB, in which the additional AUG (ATG in DNA) had been deleted, could be more efficiently translated into h-IFN-beta protein. The significance of this PR assay is that it showed that the construction of pMTIF-betaA and B is correct, that the plasmids could be expressed by mouse L cells, that the h-IFN-beta protein could be secreted from the cell. So, in theory, if these plasmids (or the MT-IFN fragments of these plasmids) are microinjected into mouse eggs and transgenic mice are generated, h-IFN-beta gene should also be expressible.

The 96-well microtiter CPE assay uses the same principle as the PR assay, and showed parallel results. First, 100 ul medium (DMEM plus 15% calf serum) was added to each well except for "#1" wells (first whole column). Then samples and medium were added to the #1 wells so each well contained 200 ul total. After completely mixing the samples in the #1 wells with medium, transfer 100 ul solution from each first well to the second well (that is, to make a 2X dilution), and then transfer to the third well, and so forth, until the last well is reached. Then add to each well 100 ul of human WISH cells (3 x 10^4 cells/100 ul) and incubate the cell-sample mixtures 6 hours to overnight. After incubation, add 50 ul VSV (3 x 10^3 PFU/50 ul) to challenge the cells in each well (except cell control wells), and incubate for 48 hours before staining. Next, stain with crystal violet and wash excess crystal violet stain away. Compare sample wells with IFN standards and controls of cells and cell-virus to determine protection and killing. The concentration of
h-IFN-beta in the samples is determined on the basis that one unit of interferon is defined as the amount of interferon in a sample (at a certain dilution) which can give 50% protection to WISH cells against killing by VSV. In other words, the concentration of h-IFN-beta in the original sample is the reciprocal of the dilutions of a sample which showed 50% protection. CPE assays showed consistent results with PR, i.e., media from cell transfected with pMTIF-B had consistently higher activity than media from cells transfected with pMTIF-A.

Since the PR and CPE assays clearly showed interferon expression from cells transfected with MTIF-beta A and B demonstrating the functionally of these fusion genes to produce interferon in the mouse system, analyses were carried out on serum from transgenic mice containing MTIF-beta A and B to determine if these mice were producing interferon and specifically human interferon. The CPE assay was used to analyze the MTIF-beta mouse serum. The transgenic mice with the MTIF-beta A gene expressed ~50 units of interferon per ml of serum, while the MTIF-beta B transgenic mice showed a level of interferon 3-5 fold higher. This difference in expression level is attributed to the greater efficiency of translation of the MTIF-beta RNA message in the MTIF-beta B mice resulting from the removal of the competing spurious ATG codon.

In order to confirm that the anti-viral activity of the sera of transgenic mice is due to the presence of human beta interferons, an antigen-antibody neutralization CPE assay was carried out. The difference between this assay and the standard CPE assay is simply to divide each mouse serum sample into
two, one half of the sample was added to the first column well as before; the other half was mixed with horse anti-human beta interferon antibody and then was applied to another first column well. The serial dilutions were carried out after the sample was incubated with the antibody for 30 minutes or an hour. The co-incubation with cells, VSV challenging, staining are just the same as in common CPE assay. The result demonstrated that serum from transgenic mice could protect cells from viral infection whereas serum (same serum) preincubated with the antibody could not. This is a clear indication that the antiviral activity of the mouse serum on human cells was from human beta interferon. At the same time, negative serum control and antibody themselves could not protect the cells, which suggests that this antiviral activity is, and only is, from human beta interferon expressed in transgenic mice.

Example 4: Viral Resistance of Transgenic Mice

Transgenic mice containing the MTIF-beta A gene and mice containing the MTIF-beta A gene were produced as described in Example 2 and characterized as in Example 3. To evaluate the viral resistance of these mice, groups of mice were exposed to several concentrations of pseudorabies (RPV) (Rice strain 2-10-86), a virus lethal to mice, by footpad injection. Mortality was then compared with similarly inoculated controls. The results of this virus resistance assay are presented in Figure 4. When mice were inoculated with increasing doses (p.f.u.) of the pseudorabies virus transgenic mice expressing human interferon beta consistently showed decreased mortality. At 30 p.f.u., 2 of 5 control mice died (40%) while only 1 of 5 MTIF-A
transgenic mice died (20%). At 60 p.f.u. 4 of 5 control mice died (80%) while only 1 of 5 MTIF-Beta A transgenic mice died (20%). At 86 p.f.u. 15 of 15 control mice died (100%) while only 2 of 4 MTIF-beta B transgenic mice died (50%) and the human interferon gene-bearing transgenic mice died considerably later than did the control animals. When MTIF-beta A transgenic mice were challenged with the same dose of pseudorabies virus (86 p.f.u.) all of these mice died, but they died much later than did the control mice. The increased resistance shown by the MTIF-beta B mice is attributed to the 3-5 fold higher level of human interferon present within these animals. The mice used as controls were the same isogeneic F1 strain of mice as the transgenic mice, differing only in the presence of MTIF-beta DNA sequences in their chromosomal composition. These results are consistent with a finding that human interferon-expressing mice are virus resistant.

Example 5: Proposed Protocol For the Production of Swine With Human Beta Interferon Gene

Swine containing the human interferon beta gene are constructed by microinjecting the MTIF-beta fusion gene into the one cell fertilized egg of the pig, transferring the developing embryo into a recipient female pig which ovulated at approximately the same time as the sow used to donate the fertilized eggs, and allowing the development of these eggs to term. When the pigs resulting from the microinjected eggs are born tissue samples are taken and analyzed for the presence of the MTIF-beta gene by hybridization analysis as described in Example 2 for the mouse. Pigs containing the MTIF-beta gene are then analyzed for expression of
human beta interferon using the assays described in Example 3. Pigs expressing human beta interferon are then challenged with a virus, such as pseudorabies virus, to determine their resistance to viral infection.
CLAIMS

1. A transgenic, non-human vertebrate animal of a first animal species, having one or more cells bearing a gene coding on expression for an interferon of a second animal species, which interferon has antiviral activity.

2. The animal of claim 1, where said gene is a human interferon gene.

3. The animal of claim 1, where said gene is a beta interferon gene.

4. The animal of claim 1, wherein said gene is operably connected to an inducible promoter.

5. The animal of claim 1, wherein the first species is mammalian.

6. The animal of claim 5, wherein the first species is selected from the group consisting of cows, goats, sheep, pigs, horses, dogs and cats.

7. A method of producing an animal according to claim 1 which comprises providing an interferon gene of said second species operably linked to a promoter functional in a host animal of said first species, and introducing said gene into one or more cells of the host animal in such a manner that it will be replicated by said cells.

8. The method of claim 7 in which the gene is microinjected into one or more cells of said host animal, the host animal being at an embryonic
stage of development.

9. The method of claim 7 in which the gene is introduced into germ line cells of the host animal.

10. The method of claim 7 in which the promoter is an inducible promoter.

11. The method of claim 10 in which the induction is tissue-specific.

12. The method of claim 10 in which the induction is development-specific.

13. The method of claim 7 in which the level of expression of said gene is such that the interferon expressed would be toxic to the host if said interferon were of said first species.

14. A method of protecting an animal against viral infection which comprises introducing a gene coding an expression for an interferon species not native to the host animal into one or more cells of a host animal, said gene being operably linked to a promoter functional in said host animal, and providing conditions under which said gene will express an interferon having antiviral activity against said infection.

15. The method of claim 14 wherein said infection is selected from the group consisting of infectious bovine rhinotracheitis, parainfluenza, bovine viral diarrhea, rabies, foot and mouth disease, TGE, mycoplasmal pneumonia, pseudorabies, Marek’s
disease, Newcastle disease, infectious bursal disease, and infectious bronchitis.

16. The method of claim 14 wherein the infection is caused by a pseudorabies virus.

17. The method of claim 14 wherein the gene is replicated by said cells, thereby maintaining the antiviral resistance of the animal.

18. The method of claim 14 in which the expression is induced before the onset of the viral infection.

19. The method of claim 14 in which the protected animal is a descendant of the host animal to which said gene has been transmitted by genetic inheritance.

20. The method of claim 14, further comprising immunotherapy or immunoprophylaxis of said animal.

21. The animal of claim 1, wherein said gene is operably connected to a promoter which is at least partially constitutive.

22. The method of claim 7, wherein the promoter is at least partially constitutive.

23. The method of claim 14, in which the gene is expressed essentially all of the time, thereby maintaining viral resistance.

24. The method of claim 14 in which the expression product of the gene is less active than the interferon endogenous to the host animal.
FIG. 1.

CONSTRUCTION OF pMTIF - βA

pMK 8.4kb

EcoR I

Kpn I

Bgl II

Bgl II restriction

EcoR I

MT-I promoter

Klenow filling in

EcoR I

blunt

EcoR I

pMTIF-β1A

Hind III

EcoR I

Nco I

Pvu II

Nco I

Bgl II

Hind III

Nco I

EcoR I

Pvu II

Nco I

Hind III

klenow filling in

EcoR I

blunt end

EcoR I

Hind III

EcoR I

Hind III

MT-I promoter

β-IFN 6.5kb

Bgl II

Hind III

Nco I

STICKY/BLUNT

LIGATION

SUBSTITUTE SHEET
FIG. 2.

SUBSTITUTE SHEET
FIG. 4.

A
(30 p.f.u.)

B
(60 p.f.u.)

C
(86 p.f.u.)

- CONTROL MICE
- MTIF-BETA A TRANSGENIC MICE
- MTIF-BETA B TRANSGENIC MICE

NUMBER OF MICE SURVIVING

DAYS POST-INFECTION

SUBSTITUTE SHEET
# INTERNATIONAL SEARCH REPORT

**International Application No.** PCT/US88/02770

## I. CLASSIFICATION OF SUBJECT MATTER

If several classification symbols apply, indicate all.

**U.S. Cl.** 435/172.3

**INT. Cl.** (4) C12N 15/00

## II. FIELDS SEARCHED

Minimum Documentation Searched.

<table>
<thead>
<tr>
<th>Classification System</th>
<th>Classification Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.Si.</td>
<td>435/172.3, 320, 811</td>
</tr>
<tr>
<td></td>
<td>935/53, 63, 70, 34</td>
</tr>
<tr>
<td></td>
<td>800/1</td>
</tr>
</tbody>
</table>

Documentation Searched other than Minimum Documentation to the extent that such Documents are Included in the Fields Searched.

## III. DOCUMENTS CONSIDERED TO BE RELEVANT

### Category *

<table>
<thead>
<tr>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>X</strong> US, A, 4,276,282 (SUGIMOTO) 30 June 1981, see example A-2.</td>
<td>1-7, 10-18 and 20-24</td>
</tr>
<tr>
<td><strong>X</strong> Chemical Abstracts, Volume 100 No. 15, 09 April 1984 (Columbus, Ohio, USA) Gordon, &quot;Studies of Foreign Genes, Transmitted Through the Germ Lines of Transgenic Mice&quot; see page 137, abstract No. 115805s, Journal of Experimental Medicine 228(2) p. 313-24, 1983.</td>
<td>1,2,4,5, 7-12, 21 and 22 3,6,13-20 and 24</td>
</tr>
</tbody>
</table>

* Special categories of cited documents:

  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier document but published on or after the international filing date
  - "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)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed

  "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

  "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

  "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

  "A" document member of the same patent family

## IV. CERTIFICATION

**Date of the Actual Completion of the International Search** 23 November 1988

**Date of Mailing of this International Search Report** 12 JAN 1989

**International Searching Authority** ISA/US

**Signature of Authorized Office**

[Signature]

**John E. TARCZA**
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>Nature 258, 08 November 1975, GRESSER et al, &quot;Lethality of Interferon Preparations for Newborn Mice&quot;, pages 76-78.</td>
<td>13</td>
</tr>
</tbody>
</table>