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- (73) Patenthaver: **Bavarian Nordic A/S, Hejreskovvej 10 A, 3490 Kvistgård, Danmark**
- (72) Opfinder: **CHAPLIN, Paul, Bürgerstrasse 9, 81925 München, Tyskland**
MATEO, Luis, c/o 20 Tucker St., Chapel Hill, QLD 4069, Australien
- (74) Fuldmægtig i Danmark: **RWS Group, Europa House, Chiltern Park, Chiltern Hill, Chalfont St Peter, Bucks SL9 9FG, Storbritannien**
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DESCRIPTION

[0001] The present invention relates to the rapid induction of a protective immune response against smallpox infections by vaccination of an animal, including a human with a poxvirus that is replication incompetent in said animal including the human. The invention further relates to the use of a recombinant poxvirus that is replication incompetent in the animal, including the human that is vaccinated with the virus expressing heterologous antigens and/or antigenic epitopes for a rapid induction of an protective immune responses against said heterologous antigen and/or antigenic epitope, e.g. against an antigen and/or antigenic epitope that is part of an infectious agent.

Background of the invention

[0002] For many diseases, such as infectious diseases vaccines have been developed or are in the process of being developed. These vaccines induce a protective immune response within a certain time frame. Since most vaccines are used for the vaccination against diseases that are rather rare in the population there is usually no need that the generation of the immune response is particularly rapid. However, there are situation in which an immune response, such as a protective immune response should be generated as fast as possible. This may be the case in an outbreak of smallpox or any other human poxvirus disease.

[0003] The causative agent of smallpox is the variola virus, a member of the genus Orthopoxvirus. Vaccinia virus, also a member of the genus Orthopoxvirus in the family Poxviridae, was used as live vaccine to immunize against smallpox. Successful worldwide vaccination with Vaccinia virus culminated in the eradication of variola virus (The global eradication of smallpox. Final report of the global commission for the certification of smallpox eradication; History of Public Health, No.4, Geneva: World Health Organization, 1980). In the meantime, most of the stocks of infectious variola viruses have been destroyed. However, it cannot be excluded that poxviruses inducing smallpox or smallpox-like diseases might again become a major health problem. In addition, there is a risk that a poxvirus disease of animals is spread to humans.

[0004] US 2003/202988 discloses a prime-boost immunization regimen in which vaccinia virus is administered to test mice. Three weeks after prime immunization the mice were boost immunized, and two weeks after boost immunization the mice received a challenge with a replication competent vaccinia virus. Thus, the mice were challenged two weeks after the second (boost) and five weeks after the first (prime) vaccination.

[0005] There may be situations in which it is desirable to induce a rapid immune response, e.g. it might be desirable to induce a rapid immune response against diseases which are endemic in some parts of the world if it is necessary to travel to such a country at short notice.

Detailed description of the invention and disclosure made

[0006] The present invention relates to a Modified Vaccinia virus Ankara (MVA) for use in a method of immunizing an animal, including a human, to protect against smallpox infection within 2-7 days after immunization, wherein a protective immune response is generated against smallpox within 2-7 days after immunization, and wherein the MVA is MVA-BN.

[0007] The term "replication incompetent poxvirus" and the synonymous term virus that is "not capable of being replicated to infectious progeny virus" refer to poxviruses that do not replicate at all in the cells of the vaccinated animal and to viruses that show a minor residual replication activity that is controlled by the immune system of the animal, including the human to which the poxvirus is administered.

[0008] The replication incompetent poxviruses are viruses that are capable of infecting cells of the animal, including the human in which the virus is used as vaccine. Viruses that are "capable of infecting cells" are viruses that are capable of interacting with the host cells to such an extent that the virus or at least the viral genome becomes incorporated into the host cell. Although the viruses used are capable of infecting cells of the vaccinated animal, including a human, they are either not capable of being replicated to infectious progeny virus in the cells of the vaccinated animal or they show only a minor residual replication activity that is controlled by the immune system of the animal, including the human to which the poxvirus is administered.

[0009] It is to be understood, that a virus that is capable of infecting cells of a first animal species but not capable of being replicated to infectious progeny virus in said cells may behave differently in a second animal species. E.g., for humans MVA-BN and its derivatives (see below) are viruses that are capable of infecting cells of the human but that are not capable of being replicated to infectious progeny virus in human cells. The same viruses are very efficiently replicated in chickens; i.e. in chickens MVA-BN is a virus that is capable of infecting cells and capable of being replicated to infectious progeny virus. It is known to the person skilled in the art which virus has to be chosen for a specific animal species. A test that allows determining whether a virus is capable or not capable of being replicated in mice is disclosed in WO 02/42480 and uses the AGR129 mice strain (see below) any other mouse strain that is incapable of producing mature B and T cells and as such is severely immune compromised and highly susceptible to a replicating virus. The results obtained in this mice model are indicative for humans.

[0010] The viruses are capable of being replicated in at least one type of cells of at least one animal species. Thus, it is possible to amplify the virus prior to administration to the animal that is to be vaccinated and/or treated. By way of example reference is made to MVA-BN that can be amplified in CEF (chicken embryo fibroblasts) cells but that is a virus that is not capable of being replicated to infectious progeny virus in humans.

[0011] Modified Vaccinia virus Ankara (MVA) is used in humans and several animal species such as mice and non-human primates. MVA is known to be exceptionally safe. MVA has been generated by long-term serial passages of the Ankara strain of Vaccinia virus (CVA) on chicken embryo fibroblasts (for review see Mayr, A., Hochstein-Mintzel, V. and Stickl, H. [1975] Infection 3, 6-14; Swiss Patent No. 568, 392). Examples for MVA virus strains that have been deposited in compliance with the requirements of the Budapest Treaty and that are useful in the practice of the present invention are strains MVA 572 deposited at the European Collection of Animal Cell Cultures (ECACC), Salisbury (UK) with the deposition number ECACC 94012707 on January 27, 1994, MVA 575 deposited under ECACC 00120707 on December 7, 2000 and MVA-BN deposited with the number 00083008 at the ECACC on August 30, 2000.

[0012] According to the present invention the MVA strain is MVA-BN and its derivatives. A definition of MVA-BN and its derivatives is given in PCT/EP01/13628.

[0013] In short, MVA-BN and its derivatives as disclosed in PCT/EP01/13628 are characterized in having at least one, at least two, at least three or all of the following properties:

1. (i) capability of reproductive replication in chicken embryo fibroblasts (CEF) and in the cell line BHK, but no capability of reproductive replication in human cell lines. According to an embodiment of the present invention the human cell lines are the human bone osteosarcoma cell line 143B, the human keratinocyte cell line HaCat and the human cervix adenocarcinoma cell line HeLa,
2. (ii) failure to replicate in vivo in severely immune compromised mice,
3. (iii) induction of a higher immunogenicity compared to the known strain MVA 575 (ECACC V00120707) in a lethal challenge model and/or
4. (iv) induction of at least substantially the same level of immunity in vaccinia virus prime/ vaccinia virus boost regimes when compared to DNA-prime/ vaccinia virus boost regimes.

[0014] For detailed information regarding to the assays used to determine whether an MVA strain has one or more of the above features (i) to (iv) reference is made to WO 02/42480 (PCT/EP01/13628). This publication also discloses how viruses having the desired properties can be obtained. In the following it is shortly summarized how the person skilled in the art can test whether an MVA strain has one or more of said features and is, thus, a virus according to said embodiment of the present invention. The following summary is not to be understood as to limit the relevance of WO 02/42480 for the present application to the following information. The term "not capable of reproductive replication" in human cell lines such as the cell lines HaCAT (Boukamp et al. 1988, J Cell Biol 106(3): 761-71) or HeLa is used in the present application as defined in WO 02/42480. Thus, a virus that is "not capable of reproductive replication" in a cell line is a virus that shows an amplification ratio of less than 1 in said cell line. The "amplification ratio" of a virus is the ratio of virus produced from an infected cell (Output) to the amount originally used to infect the cells in the first place (Input). A ratio of "1"

between Output and Input defines an amplification status wherein the amount of virus produced from the infected cells is the same as the amount initially used to infect the cells. According to the present invention the viruses that are "not capable of reproductive replication" in human cell lines may have an amplification ratio of 1.0 (average value) or less or even 0.8 (average value) or less in any of the above human cell lines HeLa, HaCat and 143B

[0015] The term "average" as used in the present application refers to the average values obtained from of at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or more experiments. It will be understood by a person skilled in the art that single experiments may deviate from average values due to the inherent variability of biological systems.

[0016] The term "failure to replicate in vivo" is used in the present application as defined in WO 02/42480. Thus, said term refers to viruses that do not replicate in the mice model as explained in WO 02/42480. The mice used in WO 02/42480 are incapable of producing mature B- and T-cells (AGR 129 mice). MVA-BN and its derivatives do not kill AGR129 mice within an average time period of at least 45 days (average value), such as within at least 60 days (average value), or within 90 days (average value) after the infection of the mice with 10^7 pfu virus administered intra peritoneally. According to an embodiment of the present invention, the viruses that show "failure to replicate in vivo" are further characterized in that no virus can be recovered from organs or tissues of the AGR129 mice 45 days (average value), alternatively 60 days (average value) and alternatively 90 days (average value) after the infection of the mice with 10^7 pfu virus administered intra peritoneally. Instead of the AGR129 mice any other mouse strain can be used that is incapable of producing mature B and T cells and as such is severely immune compromised and highly susceptible to a replicating virus. The data obtained in said mice model are predictive for humans. Thus, the viruses of the present invention, such as MVA-BN and its derivatives, do not replicate at all in humans. In applying the definition in the section related to the terms "replication incompetent poxvirus" and "virus that is not capable of being replicated to infectious progeny virus" to the replication behaviour of MVA-BN and its derivatives in humans also those viruses are within the scope of the present invention that show a minor residual replication activity that is controlled by the immune system of the human to which the poxvirus is administered.

[0017] The details of the lethal challenge experiment used to determine whether an MVA strain has "a higher immunogenicity compared to the known strain MVA 575" are explained in WO 02/42480. In such a lethal challenge model unvaccinated mice die after the infection with replication competent vaccinia strains such as the Western Reserve strain L929 TK+ or IHD-J. The infection with replication competent vaccinia viruses is referred to as "challenge" in the context of description of the lethal challenge model. Four days after the challenge the mice are usually killed and the viral titer in the ovaries is determined by standard plaque assays using VERO cells. The viral titer is determined for unvaccinated mice and for mice vaccinated with MVA-BN and its derivatives. More specifically MVA-BN and its derivatives are characterized in that in this test after the vaccination with 10^2 TCID₅₀/ml virus the ovary virus titers are reduced by at least 70% (average value), alternatively by at least 80% (average value), alternatively by

at least 90% (average value) compared to unvaccinated mice.

[0018] A vaccinia virus, such as an MVA strain is regarded as inducing at least substantially the same level of immunity in vaccinia virus prime/ vaccinia virus boost regimes when compared to DNA-prime/ vaccinia virus boost regimes if the CTL response as measured in one of the "assay 1" and "assay 2" as disclosed in WO 02/42480 is at least substantially the same in vaccinia virus prime/ vaccinia virus boost regimes when compared to DNA-prime/ vaccinia virus boost regimes. According to an embodiment of the present invention the CTL response is at least substantially the same in vaccinia virus prime/ vaccinia virus boost regimes when compared to DNA-prime/ vaccinia virus boost regimes as measured in both of the "assay 1" and "assay 2" as disclosed in WO 02/42480. According to an embodiment of the present invention the CTL response after vaccinia virus prime/vaccinia virus boost administration is higher in at least one of the assays, when compared to DNA-prime/vaccinia virus boost regimes. According to an embodiment of the present invention the CTL response is higher in both assays.

[0019] According to the present invention the derivatives of MVA-BN are characterized (i) in being capable of reproductive replication in chicken embryo fibroblasts (CEF) and the Baby hamster kidney cell line BHK but not capable of reproductive replication in human cell lines, wherein according to an embodiment of the present invention the human cell lines are the human bone osteosarcoma cell line 143B, the human keratinocyte cell line HaCat and the human cervix adenocarcinoma cell line HeLa and (ii) by a failure to replicate in vivo in severely immune compromised mice.

[0020] According to an embodiment of the present invention the virus is a clone purified virus, such as a monoclonal virus.

[0021] According to an embodiment of the present invention the virus is a virus that has been produced/passaged under serum free conditions to reduce the risk of infections with agents contained in serum.

[0022] MVA-BN is administered in a concentration range of 10^4 to 10^9 TCID₅₀/ml, e.g. in a concentration range of e.g. 10^5 to 5×10^8 TCID₅₀/ml or in a concentration range of e.g. 10^6 to 10^8 TCID₅₀/ml. The actual concentration depends on the type of the virus and the animal species to be vaccinated. For MVA-BN a typical vaccination dose for humans comprises 5×10^7 TCID₅₀ to 5×10^8 TCID₅₀, e.g. about 1×10^8 TCID₅₀, administered subcutaneously.

[0023] According to the present invention the poxvirus as defined above, i.e. the MVA-BN and its derivatives is administered in a single administration to induce a rapid a protective immune response. Clinical data have shown that a single vaccination with MVA-BN resulted in a detectable immune response in almost 100% of the vaccinated individuals.

[0024] According to the present invention the poxvirus as defined above, i.e. MVA-BN and its

derivatives may also be used in homologous prime boost regimes, i.e. it is possible to use a MVA for a first vaccination and to boost the immune response generated in the first vaccination by administration of the same or a related strain of the MVA than the one used in the first vaccination. The poxvirus as defined above, i.e. MVA-BN and its derivatives may also be used in heterologous prime-boost regimes in which one or more of the vaccinations is done with a poxvirus as defined above and in with one or more of the vaccinations is done with another type of vaccine, e.g. another virus vaccine, a protein or a nucleic acid vaccine.

[0025] The mode of administration may be intravenously, intradermal, intranasal, or subcutaneously. Any other mode of administration may be used such as scarification.

[0026] The poxvirus used according to the present invention may be a non-recombinant poxvirus such as MVA-BN and its derivatives. In this case the vaccination may be done to rapidly induce a protective immune response against smallpox. Thus, according to the present invention the poxvirus as defined above, i.e. MVA-BN and its derivatives is suitable to rapidly induce of a protective immune response against smallpox. This is exemplified in Example 1, where it is compared how long it takes until a protective immune response is generated in mice against a pathogenic vaccinia virus strain after vaccination with MVA-BN (a strain according to the present invention) and non-MVA strains such as Dryvax and Elstree. These strains are fully replication competent, in contrast to MVA-BN. It is shown that MVA-BN clearly has improved properties compared to Elstree and Dryvax in that a single vaccination of mice with MVA-BN leads to a significant protective immune response within four, three and even two days. This is demonstrated, e.g. by assessing in the lungs the titre of a pathogenic Vaccinia virus strain Western reserve (VV-WR) administered two, three or four days after the vaccination with MVA-BN. When the mice were challenged with $12.5 \times \text{MLD}_{50}$ of VV-WR three days after the vaccination no VV-WR viral titre could be detected in mice vaccinated with a standard dose of MVA-BN, whereas the mice vaccinated with Dryvax or Elstree were not protected and had a lung titre that was very similar the titre of unvaccinated control mice. When the mice were challenged with $50 \times \text{MLD}_{50}$ four days after the vaccination no VV-WR viral titre could be detected in mice vaccinated with a standard dose of MVA-BN, whereas the mice vaccinated with Dryvax or Elstree were not protected and had a lung titre that was very similar the titre of unvaccinated control mice. The term MLD_{50} refers to the concentration of a pathogenic Vaccinia virus strain at which 50% of the inoculated mice die.

[0027] It is to be noted that the mice data are predictive for humans. Moreover, it is to be taken into account that concentrations of a pathogenic virus that are 50 times the lethal dose usually do not occur in nature, in particular not for human poxviruses that induce smallpox.

[0028] According to the present invention the term "rapid induction of a protective immune response in an animal, including a human" refers preferably to the generation of a protective immune response within 2-7 days after immunization, preferably within 6 days or less, 5 days or less, 4 days or less, or 3 days or less after the vaccination with a virus according to the present invention. This is unexpected since it was a dogma in the state of the art that it takes at

least 10 to 14 days until a protective immune response is generated against traditional smallpox vaccines, based on replicating vaccinia virus strains. The rapidity of the induction of a protective immune response can be evaluated in the animal model described in the example section. Said model is also predictive for humans. Thus, according to an embodiment of the present invention a poxvirus vaccine is effective in inducing a rapid immune response in mice if after vaccination of mice with an effective dose of a poxvirus vaccine, i.e. MVA-BN and derivatives thereof, and challenge with 1x, 12.5x and 50, MLD₅₀, respectively of VV-WR four days after vaccination the lung titres of the virus are below an average of 5×10^3 pfu (corresponding to log 3.69) as determined in the test system described in the example section. Alternatively the values are below an average of 5×10^3 pfu (corresponding to log 3.69) after an challenge with 1 x, and 12.5x MLD₅₀, respectively, of VV-WR three days after vaccination with an effective dose of the poxvirus vaccine. In a broader sense a virus leads to a rapid induction of a protective immune response in mice if said virus behaves similar to MVA-BN in the lung titre assay and the body weight assay described in the example. Thus, the limits, threshold values, conditions and parameters as described in the example section also apply in a general sense for other poxvirus vaccines that are regarded as rapid inducers of a protective immune response. From this it is obvious that the data and information given in the example section can be generally used to supplement any missing data and information in this paragraph, e.g. information relating to the description of the test system.

[0029] Alternatively the rapidity of the induction of the protective immune response can be evaluated with the serum conversion test explained below; in this context the time point at which seroconversion is observed is regarded as the time point at which the protective immune response was induced.

[0030] According to an embodiment of the present invention the animal, including a human is an animal, including a human that is naïve with respect to poxvirus infections, i.e. an animal, including a human, that has never been in contact with poxviruses and that has not been vaccinated with poxvirus vaccines.

[0031] According to a related embodiment the animal, including a human is an animal, including a human that was in contact with poxviruses and/or that was vaccinated with a poxvirus vaccine. These animals, including a human, might have raised an immune response against poxviruses and/or poxvirus vaccines such as MVA.

[0032] The term "protective immune response" means that the vaccinated animal is able to control an infection with the pathogenic agent against which the vaccination was done. Usually, the animal having developed a "protective immune response" develops only mild to moderate clinical symptoms or no symptoms at all. Usually an animal having a "protective immune response" against a certain agent will not die as a result of the infection with said agent.

[0033] As pointed out above a concentration of MVA-BN or a derivative thereof for the generation of a protective immune response in humans against smallpox is in the range of $5 \times$

10^7 TCID₅₀ to 5×10^8 TCID₅₀, such as 1×10^8 TCID₅₀, wherein the virus may be administered subcutaneously or intramuscularly.

[0034] It seems as if the mechanism of the development of a rapid immune protection after vaccination with a poxvirus as defined above, i.e. MVA-BN and its derivatives depends on whether the vaccinated animal including a human is a naïve animal (that was never in contact with a poxvirus before) or an animal that had been in contact with a poxvirus before (e.g. by vaccination). In the naïve animal, including a human the administration of the poxvirus according to the present invention, i.e. MVA-BN or its derivatives efficiently primes the immune system, even if neutralizing antibodies may not be detectable in the first few days after vaccination. The infection with a pathogenic virus boosts the immune system in such a way that the effectively primed immune system can control said infection unexpectedly effective and fast. Thus, naïve animals that have been vaccinated with a virus according to the present invention are readily protected against the infection with the pathogenic virus against which the vaccination is done after a single vaccination only.

[0035] The viruses as defined according to the present invention, i.e. MVA-BN and its derivatives also are unexpectedly efficient and fast in boosting the earlier vaccination in animals that have been in contact with a poxvirus before so that a protective immune response is also rapidly generated in this situation.

[0036] The rapidity of the induction of a protective immune response is also reflected by an unexpectedly fast seroconversion after vaccination of animals, including humans with a virus according to the present invention, i.e. MVA-BN and its derivatives. In non-human primates is shown that a seroconversion occurs within less than 10 days, e.g. within 7 days which is one week faster than the seroconversion after vaccination with other smallpox vaccines such as Elstree. In the following it is described how the seroconversion after vaccination with MVA-BN and its derivatives is evaluated. The same test principle is applied mutatis mutandis if the seroconversion after vaccination with other viruses is tested. The only modification that is required to assess the seroconversion induced by said other virus is to quantify the total IgG antibodies specific for said other virus instead of quantifying the total MVA-BN specific IgG antibodies. The cut off values and the criteria to evaluate whether a sample is positive are determined in basically the same way with optional minor modifications that are within the skills of the skilled artisan. To assess for a seroconversion after vaccination with MVA-BN (or its derivatives), total MVA-BN[®] specific IgG antibodies are quantified in test sera using a direct ELISA. The ELISA is a sensitive method used for the detection of antibodies in sera. The MVA-BN[®] specific ELISA is a standard binding ELISA used to detect total IgG antibodies in human test sera. ELISA results are expressed as an end point antibody titre obtained by direct determination of logarithmic trend lines. A cut off, or end point absorbance of 0.35 has been defined. The end point titre of the sample is determined by generating a logarithmic plot, e.g. by using the commercially available computer program Excel (optical density (OD) on the y axis and the log of the sera dilution on the x axis). Again the data in non-human primates are predictive for humans. A test sample is deemed positive when the OD of the sample is greater

than 0.35 at a 1:50 dilution of a test sample. The geometric mean titre (GMT) is calculated by taking the antilogarithm of the mean of the logs 10 titre transformations. The GMT is usually the reported titre for ELISA titres. Seroconversion rate is defined as percentage of initially seronegative subjects with appearance of antibody titres $\geq 1:50$ in the MVA specific IgG ELISA.

[0037] The poxvirus as defined above, i.e. MVA-BN and its derivatives may also be a recombinant poxvirus strain such as recombinant MVA-BN or its derivatives. The recombinant viruses according to the present invention, such as recombinant MVA-BN and its derivatives may contain at least one heterologous nucleic acid sequence. The term "heterologous" is used hereinafter for any combination of nucleic acid sequences that is not normally found intimately associated with the virus in nature. The heterologous sequences may be antigenic epitopes or antigens, which are selected from any non-vaccinia source. According to an embodiment of the present invention, said recombinant virus expresses one or more antigenic epitopes or antigens which are antigenic epitopes or antigens from an infectious agent. The infectious agent may be any infectious agent such as a virus, a fungus, a pathogenic unicellular eukaryotic and/or prokaryotic organism or a parasitic organisms. Examples for infectious agents are Plasmodium falciparum, Mycobacteria, Influenza virus, Flaviviruses, Paramyxoviruses, Hepatitis viruses, Human immunodeficiency viruses, viruses causing hemorrhagic fever such as Hantaviruses or Filoviruses, i.e., Ebola or Marburg virus.

[0038] According to this embodiment the recombinant poxvirus as defined above may not only be used to induce a rapid immune response against a smallpox infection but may also (or alternatively) be used to induce a rapid immune response against the heterologous antigenic epitope/ antigen expressed from the heterologous nucleic acid comprised in the recombinant virus. Thus, by way of example if a recombinant MVA expresses an HIV epitope or a yellow fever virus epitope the recombinant MVA may be used to induce a rapid immune response against HIV and Yellow fever virus, respectively.

[0039] It is also within the scope of the present invention that the recombinant virus may alternatively express an antigenic epitope/ antigen that further increases the immunogenicity of MVA.

[0040] The recombinant virus used according to the present invention may also comprise a heterologous gene/nucleic acid expressing a therapeutic compound. A "therapeutic compound" encoded by the heterologous nucleic acid in the virus can be, e.g., a therapeutic nucleic acid such as an antisense nucleic acid or a peptide or protein with desired biological activity.

[0041] According to an embodiment of the present invention the expression of heterologous nucleic acid sequence may be under the transcriptional control of a poxvirus promoter. An example of a suitable poxvirus promoter is the cowpox ATI promoter (see WO 03/097844).

[0042] According to an embodiment of the present invention the insertion of heterologous nucleic acid sequence is done into a non-essential region of the virus genome. According to another embodiment of the invention, the heterologous nucleic acid sequence is inserted at a

naturally occurring deletion site of the MVA genome (disclosed in PCT/EP96/02926). According to a further alternative the heterologous sequence may be inserted into an intergenic region of the poxviral genome (see WO 03/097845). Methods how to insert heterologous sequences into the poxviral genome are known to a person skilled in the art.

[0043] All definitions given above for the embodiment regarding non-recombinant viruses apply also for the embodiment concerning recombinant viruses.

Summary of the invention:

[0044] The invention is the following:

1. 1. A Modified Vaccinia virus Ankara (MVA) for use in a method of immunizing an animal, including a human, to protect against smallpox infection within 2-7 days after immunization, wherein a protective immune response is generated against smallpox within 2-7 days after immunization, and wherein the MVA is MVA-BN.
2. 2. The MVA of item 1, wherein the generation of a protective immune response is within 6 days or less, 5 days or less, 4 days or less, or 3 days or less.
3. 3. The MVA of item 1 to 2, wherein the virus is a clone purified virus.
4. 4. The MVA of item 1 to 3, wherein the virus has been obtained in a serum free cultivation process.
5. 5. The MVA of item 1 to 4, wherein the virus is administered in a dose of 10^5 to 5×10^8 TCID₅₀/ml.
6. 6. The MVA of item 1 to 5, wherein the virus is administered intravenously, intramuscularly or subcutaneously.
7. 7. The MVA of item 1 to 6, wherein the virus is administered subcutaneously.
8. 8. The MVA of item 1 to 7, wherein the virus is a recombinant virus.
9. 9. The MVA of item 8, wherein the recombinant virus comprises at least one heterologous nucleic acid sequence.
10. 10. The MVA of item 9, wherein the heterologous nucleic acid sequence is a sequence coding for at least one antigen, antigenic epitope, and/or a therapeutic compound.
11. 11. The MVA of item 10, wherein the antigenic epitopes and/or the antigens are antigenic epitopes and/or antigens of an infectious agent.
12. 12. The MVA of item 11, wherein the antigenic agent is selected from viruses, fungi, pathogenic unicellular eukaryotic and/or prokaryotic organisms, parasitic organisms.
13. 13. The MVA of item 12, wherein the viruses are selected from the family of Influenza virus, Flavivirus, Paramyxovirus, Hepatitis virus, Human immunodeficiency virus or from viruses causing hemorrhagic fever.
14. 14. The MVA of item 1 to 13, wherein the generation of a protective immune response is reflected by a seroconversion.

Short description of the figures:**[0045]**

Figure 1: Changes in body weight of differently vaccinated mice following an intranasal challenge with 1x the MLD₅₀ of VV-WR. BALB/c mice were vaccinated subcutaneously with MVA-BN[®], Elstree, Dryvax, or with Saline (PBS) 4 days, or with MVA-BN[®] 3 days or 2 days prior to challenge with 4×10^4 TCID₅₀ of VV-WR per mouse. Body weight was measured prior to challenge (day 0) and then daily post challenge at the same time each day. The graph represent the average body weight changes per group over time using normalized (day 0 as baseline value) individual values.

Figure 2: Changes in body weight of differently vaccinated mice following an intranasal challenge with 12.5x the MLD₅₀ of VV-WR. BALB/c mice were vaccinated subcutaneously with MVA-BN[®], Elstree, Dryvax, or with Saline (PBS) 4 days, or with MVA-BN[®] 3 days prior to challenge with 5×10^5 TCID₅₀ of VV-WR per mouse. Body weight was measured prior to challenge (day 0) and then daily post challenge at the same time each day. The graph represent the average body weight changes per group over time using normalized (day 0 as baseline value) individual values.

Figure 3: Changes in body weight of differently vaccinated mice following an intranasal challenge with 50x the MLD₅₀ of VV-WR. BALB/c mice were vaccinated subcutaneously with MVA-BN[®], Elstree, Dryvax, or with Saline (PBS) 4 days, or with MVA-BN[®] 3 days prior to challenge with 2×10^6 TCID₅₀ of VV-WR per mouse. Body weight was measured prior to challenge (day 0) and then daily post challenge at the same time each day. The graph represent the average body weight changes per group over time using normalized (day 0 as baseline value) individual values.

Figures 4, 5 and 6: Viral load of lungs at the time of death or sacrifice following intranasal challenge with 1x the MLD₅₀ of VV-WR (figure 4), with 12.5x the MLD₅₀ of VV-WR (figure 5) or 50 x the MLD₅₀ of VV-WR (Figure 6). Lungs were collected from each animal and the viral load was determined using a standard plaque assay on Vero cells. The bars represent the average lung titre per group $\pm$ standard error.

Examples:

[0046] The following examples will further illustrate the present invention. It will be well understood by a person skilled in the art that the provided examples in no way may be

interpreted in a way that limits the applicability of the technology provided by the present invention to this examples.

Example 1: Onset of protection in MVA-BN, Elstree, or DryVax vaccinated mice challenged with 1x, 12.5x or 50x the MLD₅₀ of vaccinia virus VV-Western Reserve (VV-WR)

1. Introduction

[0047] A murine intranasal vaccinia challenge model has been developed to test the efficacy of smallpox vaccine candidates. In this model, mice are vaccinated with vaccines the efficiency of which is to be determined. Control mice receive a saline control instead of the vaccine. Several days after the vaccination the mice are infected with a pathogenic Vaccinia virus strain such as the the vaccinia virus strain Western Reserve (VV-WR). The murine lethal dose 50 (MLD₅₀) of the vaccinia virus strain Western Reserve (VV-WR) was determined to be 3.6×10^4 TCID₅₀ in unvaccinated mice.

In a preliminary study it was shown that MVA-BN vaccinated BALB/c mice challenged with either 25x or 50x the MLD₅₀ of VV-WR quickly recovered from the viral challenge, showed no clear signs of clinical symptoms, and no pathological lesions were present in the lungs of these animals. In another preliminary study the time required after vaccination to establish protection from a lethal challenge with VV-WR was investigated: Challenging of MVA-BN[®] vaccinated mice with a sub-lethal dose of VV-WR (5×10^3 TCID₅₀/mouse) 3 days after the vaccination revealed protection (with regard to body weight loss and viral lung titres). The objective of this example was to narrow down the time required after MVA-BN[®] (or Elstree or Dryvax) vaccination to obtain protection following a lethal challenge with VV-WR.

2. Viruses and controls:

Test vaccine 1

[0048] Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN[®]), in a concentration of 5.0×10^8 TCID₅₀/ml. Formulation: in 10mM Tris 140mM NaCl pH7.4

[0049] No further dilutions of the MVA-BN[®] stock were made in the MVA-BN[®] vaccinated groups and 200µl was administered subcutaneously resulting in a final dose of 1.0×10^8 TCID₅₀.

Test Vaccine 2

[0050] Vaccinia virus strain Elstree with a nominal concentration of 1.0×10^8 TCID₅₀/ml; Formulation: in 10mM Tris 140mM NaCl pH7.4

[0051] No further dilutions of the Elstree stock were made and 2.5µl was administered via scarification on the tail of each mouse resulting in a final dose of 2.5×10^5 TCID₅₀.

Test vaccine 3

[0052] DryVax with a nominal concentration of 2.9×10^7 TCID₅₀/ml; Formulation: in 10mM Tris 140mM NaCl pH7.4

No further dilutions of the DryVax stock were made and 8µl was administered via scarification on the tail of each mouse resulting in a final dose of 2.5×10^5 TCID₅₀.

[0053] Each of the test vaccines 1 to 3 was administered with its optimal dose and route of administration.

Challenge virus

[0054] Vaccinia Virus Western Reserve (VV-WR) with a nominal Concentration of 4.0×10^8 TCID₅₀/ml

[0055] The following dilutions of VV-WR (4.0×10^8 TCID₅₀/ml) were made to generate a final working stock of 8.0×10^4 TCID₅₀/ml and 4.0×10^7 TCID₅₀/ml: **For 1x the MLD₅₀ of VV-WR/mouse** (8.0×10^5 TCID₅₀/ml working stock suspension): 100µl of the VV-WR stock 4.0×10^8 TCID₅₀/ml was added to 900µl of PBS, mixed by vortexing (to give a concentration of 4.0×10^7 TCID₅₀/ml), 100µl of this suspension was transferred to 900µl of PBS, again mixed by vortexing (to give a concentration of 4.0×10^6 TCID₅₀/ml), 600µl of this suspension was added to 2400µl of PBS, mixed by vortexing to give a final concentration of 8.0×10^5 TCID₅₀/ml. **For 50x the MLD₅₀ of VV-WR/mouse** (4.0×10^7 TCID₅₀/ml working stock suspension): 300µl of the VV-WR stock 4.0×10^8 TCID₅₀/ml was added to 2700µl of PBS and mixed by vortexing to give a final concentration of 4.0×10^7 TCID₅₀/ml.

Saline Control:

[0056] In the Saline Control groups, 200µl PBS (as supplied by the manufacturer) was used for injecting individual mice subcutaneously

3. METHODS AND EXPERIMENTAL DESIGN

Test System

[0057] Specific Pathogen Free (SPF) female Balb/c mice H-2d were obtained from Taconic M&B, P.O. Box 1079, DK-8680 Ry, Denmark. Number of animals in the study: 60. Age at initiation of challenge: 9 weeks. Body weight range at initiation of challenge: 18 -23 grams. The BALB/c mouse strain has been used extensively to test the immunogenicity and efficacy of smallpox vaccines. The strain is highly susceptible to VV-WR challenge.

[0058] The experiments were carried out in accordance with the DyreforsØgstilsynet of Denmark

[0059] Allocation to treatment groups: On arrival animals were randomly allocated to a treatment group consisting of 5 animals per test group (and cage).

[0060] Justification of the dose level: MVA-BN[®] was used at an optimal dose that has been demonstrated in previous experiments to induce a strong humoral and cell mediated immune responses in mice. Elstree and Dryvax was used at a dose suggested for humans.

Vaccination and challenge schedule

[0061] A total of 80 mice were used in this study (see table below). Mice were either challenged with 1x, 12.5x, or 50x the MLD₅₀ of VV-WR 4 days after vaccination with either MVA-BN[®], or Elstree, or Dryvax. In additional groups, mice have been challenged with 1x, 12.5x, or 50x the MLD₅₀ of VV-WR 3 days after vaccination with MVA-BN[®], or mice have been challenged with 1x the MLD₅₀ of VV-WR 2 days after vaccination with MVA-BN[®]. In control groups mice have been challenged with 1x, 12.5x, or 50x the MLD₅₀ of VV-WR without prior vaccination. Control animals receiving the 1x and 12.5x, or 50x the MLD₅₀ of VV-WR were sacrificed 5 or 4 days after challenge, respectively, in case body weight loss exceeded 20% from initial body weight, or in case the animals suffered from dyspnea. This was done to reduce suffering.

Group	Vaccination	Sample Size	Challenge Dose (x the MLD ₅₀ of VV-WR)	Time between vaccination and challenge
1	Saline	5	1x	4 days
2	Saline	5	12.5x	4 days
3	Saline	5	50x	4 days
4	MVA-BN®	5	1x	4 days
5	MVA-BN®	5	12.5x	4 days
6	MVA-BN®	5	50x	4 days
7	MVA-BN®	5	1x	3 days
8	MVA-BN®	5	12.5x	3 days
9	MVA-BN®	5	50x	3 days
10	MVA-BN®	5	50x	2 days
11	DryVax	5	1x	4 days
12	DryVax	5	12.5x	4 days
14	DryVax	5	50x	4 days
15	Elstree	5	1x	4 days
16	Elstree	5	12.5x	4 days
17	Elstree	5	50x	4 days

Justification of group size

[0062] The primary endpoint of the study was efficacy determined by the level of protection at day 4 or 5 following intranasal challenge with VV-WR. Based on previous preclinical experience it was assumed that following challenge at least 95% of the vaccinated group is protected whereas no more than 5% of the placebo treated group were protected. Using Fisher's Exact test a group size of 5 versus 5 is sufficient to demonstrate a significant difference at the two-sided significance level $\alpha = 0.05$ with power greater than 80%.

Administration of test articles for vaccination

[0063] Vaccinations were performed in a microbiological safety cabinet (SW 1000 40/class II, Holten Lamin Air). Mice were vaccinated with 200µl of **MVA-BN®** (1×10^8 TCID₅₀/ml) or **Saline control** (200µl PBS) via the subcutaneous route in the skin wrinkle of the hind leg using a 1ml 29G tuberculin insulin syringe (Terumo). Mice receiving **Elstree** and **DryVax** were

anaesthetized before the scarification of the tail: A fresh mixture containing 75mg Ketamine, 5mg Xylazine and water was prepared, and 80µl of the anaesthetic was administered via the subcutaneous route using a 1ml 27G insulin syringe. All mice belonging to the one cage were anaesthetized before administering the vaccine. 2.5µl or 8µl of Elstree or Dryvax was applied via tail scarification.

Lung challenge model

[0064] The test article (i.e. VV-WR) was administered via the intranasal route in anaesthetised mice in a microbiological safety cabinet (SW 1000 40/class II, Holten Lamin Air).

[0065] A fresh mixture of 75mg Ketamine, 5mg Xylazine in water was prepared as anaesthetic. 80µl of the anaesthetic was administered via the subcutaneous route using a 1ml 29G insulin syringe. All mice belonging to one cage were anaesthetized before administering the VV-WR test article.

[0066] Intranasal challenge was performed in a microbiological safety cabinet (SW 1000 40/class II, Holten Lamin Air). The challenge virus working dilution was removed from the ice and mixed by gently vortexing for a few seconds. 50µl of the diluted VV-WR test article was measured using a 100µl pipette. The anaesthetized mouse was held by the skin/fur on the back of the neck and the body was supported in the palm of the same hand. The test item was slowly added into a single nostril of the mouse. The mouse was held as described above until the gasping ceased.

[0067] Prior to challenge (day 0) and daily following challenge animals were observed to monitor any signs of ill health. Body weight was measured prior to challenge (day 0) and daily post challenge until the day of necropsy to monitor the development of disease.

[0068] The saline group which received 50x the MLD₅₀ of VV-WR exceeded the body weight cut off set by the DyreforsØgstilsyn and was sacrificed on day 4 post challenge. The saline group which received 1x or 12.5x the MLD₅₀ of VV-WR exceeded the body weight cut off set by the DyreforsØgstilsyn and was sacrificed on day 5 post challenge. Vaccinated animals challenged with 1x the MLD₅₀ of VV-WR were sacrificed on day 5 post challenge, whereas MVA-BN[®] vaccinated animals challenged with 12.5x or 50x the MLD₅₀ of VV-WR were sacrificed latest on day 8 post challenge.

[0069] Lungs were removed and the total amount of virus in the lungs was determined using a standard plaque assay on Vero cells. Animals were considered completely protected when lung titres were below 5×10^3 pfu, the lowest titre detectable using our method of virus titration on Vero cells.

4. RESULTS AND DISCUSSION

Changes in body weight

[0070] The effect of vaccination with different smallpox vaccines on the body weight following a challenge 4 days later with 1x the MLD₅₀ of vaccinia virus strain Western Reserve (VV-WR) was investigated in some groups of this study. As shown in **Figure 1**, body weight loss in the group of non-vaccinated (Saline control) mice challenged with 1x the MLD₅₀ of VV-WR was first detectable 3 days after the challenge. The body weight continued to drop until sacrifice on day 5 to an average of 20.9% below the average initial body weight. A similar body weight loss was detected in the groups vaccinated with Elstree or Dryvax 4 days prior to challenge with VV-WR: The average body weight on day of sacrifice was either 23.2% or 21.1% below the initial average body weight in these groups, respectively. In the group vaccinated with MVA-BN[®] 2 days prior to challenge with VV-WR the first body weight loss (about 4% from average initial body weight) was detected 2 days post challenge. The average body weight continued to drop in this group until day 4 post challenge with an average body weight of 17.6% below the initial one. On day 5 post challenge, the average body weight did not continue to drop and was 16.7% below the initial average body weight. In the group vaccinated with MVA-BN[®] 3 days prior to challenge, only a small drop in body weight was detected starting on day 3 post challenge and being maximally 4.2% below the initial body weight on day 4 post challenge. In this group, the body weight recovered on day 5 to initial values. The group of mice vaccinated with MVA-BN[®] 4 days prior to challenge did not show any body weight loss following a challenge with 1x the MLD₅₀ of VV-WR.

[0071] In a second set of groups, mice were challenged with 12.5x the MLD₅₀ of VV-WR and body weight of the mice were again monitored prior to challenge and then daily post challenge. As shown in **Figure 2**, body weight loss in the group of non-vaccinated (Saline control) mice was first detectable 2 days after the challenge. The body weight continued to drop until sacrifice on day 5 to an average of 23.3% below the average initial body weight. Thus, the body weight loss was detectable one day earlier than the in the non-vaccinated group challenged with 1x the MLD₅₀ of VV-WR and was more pronounced on the day of sacrifice (see Figure 1). A body weight loss similar to the non-vaccinated mice was detected in the groups vaccinated with Elstree or Dryvax 4 days prior to challenge with VV-WR. In the group vaccinated with MVA-BN[®] 3 days prior to challenge with VV-WR a first small body weight loss (about 1.7% from average initial body weight) was detected 2 days post challenge. The average body weight continued to drop in this group until day 4 post challenge with an average body weight of 16.1 % below the initial one. Thereafter, the average body weight started to recover in this group and on day 8 post challenge an average body weight that was 2.3% below the average initial body weight was detected. In the group of mice vaccinated with MVA-BN[®] 4 days prior to challenge an average body weight loss of 10.8% compared to the average

initial body weight was detected. A maximal average body weight loss of 13.8% was detected on day 3 post challenge. Recovery of body weight was detected on the subsequent days with a similar average body weight detected 8 days post challenge than prior to challenge.

[0072] In a third set of groups, mice were challenged with 50x the MLD₅₀ of VV-WR and body weight of the mice were again monitored prior to challenge and then daily post challenge. As shown in **Figure 3**, a first body weight loss in the group of non-vaccinated (Saline control) mice was already detectable 1 day post challenge. The average body weight continued to drop until sacrifice on day 4 to 20.1% below the average initial body weight. Thus, the body weight loss was detectable 2 days or 1 day earlier than the in the non-vaccinated group challenged with 1x or 12.5x the MLD₅₀ of VV-WR, respectively. The body weight loss in the groups vaccinated with Elstree or Dryvax started to be detectable 2 days post challenge and by day 4 post challenge the mice in these groups revealed an average body weight loss of 20.1% or 19.7% from the initial body weight, respectively. In the group vaccinated with MVA-BN[®] 3 days prior to challenge with VV-WR the first body weight loss (about 1.6% from average initial body weight) was detected the first day post challenge. The average body weight continued to drop in this group until sacrifice on day 4 post challenge with an average body weight of 24.0% below the initial one. In the group of mice vaccinated with MVA-BN[®] 4 days prior to challenge a first body weight loss was detectable on the first day post challenge, the average body weight continued to drop to 22.5% below the average initial body weight on day 4 post challenge. Recovery of body weight was detected on subsequent days and on day 8 post challenge an average body weight was detected that was 5.1% below the average initial one.

Lung titres

[0073] After death or sacrifice of mice, lungs were removed and the total amount of virus in this tissue was determined using a standard plaque assay on Vero cells. Animals were considered completely protected when lung titres were below logs₁₀ 3.69 (5×10^3 pfu), the lowest titre detectable using our method of virus titration on Vero cells. In a first set of groups, mice challenged with 1x the MLD₅₀ of VV-WR were compared. As shown in **Figure 4**, non-vaccinated mice revealed an average virus load of log₁₀ 7.81. Mice vaccinated with Elstree or Dryvax 4 days prior to challenge revealed an average lung virus load of log₁₀ 7.75 and log₁₀ 6.68. Thus, the Elstree vaccinated mice were unable and the Dryvax vaccinated mice were only to some degree be able to prevent lung viral infection. In the group of mice vaccinated with MVA-BN[®] 4 days prior to challenge no lung viral titres could be detected and are thus completely protected from viral infection following intranasal challenge with 1x the MLD₅₀ of VV-WR. Shortening the interval between vaccination with MVA-BN[®] and challenge with VV-WR from 4 to 3 or 2 days increased the number of virus positive lungs per group to 1 out of 5, or 4 out of 5, with average lung viral titres of logs₁₀ 3.77 or logs₁₀ 4.68, respectively. In a second set of groups, mice challenged with 12.5x the MLD₅₀ of VV-WR were compared. As shown in **Figure 5**, non-vaccinated mice revealed an average virus load of log₁₀ 8.38. Mice

vaccinated with Elstree or Dryvax 4 days prior to challenge revealed an average lung virus load of log₁₀ 8.17 and log₁₀ 8.00. Thus, the Elstree and the Dryvax vaccinated mice were unable to prevent lung viral infection. In the group of mice vaccinated with MVA-BN[®] 4 or 3 days prior to challenge no lung viral titres could be detected and are thus completely protected from viral infection following intranasal challenge with 12.5x the MLD₅₀ of VV-WR. In a third set of groups, mice challenged with 50x the MLD₅₀ of VV-WR were compared. As shown in **Figure 6**, non-vaccinated mice revealed an average virus load of log₁₀ 8.59. Mice vaccinated with Elstree or Dryvax 4 days prior to challenge revealed an average lung virus load of log₁₀ 8.49 and log₁₀ 8.25. Thus, the Elstree and the Dryvax vaccinated mice were unable to prevent lung viral infection.

[0074] In the group of mice vaccinated with MVA-BN[®], no lung viral titres was detected when vaccination was administered 4 days prior to challenge. Consequently, these mice are protected from viral infection following intranasal challenge with 50x the MLD₅₀ of VV-WR. In the group of mice in which a 3 day interval between MVA-BN[®] vaccination and challenge with VV-WR was applied an average lung viral load of log₁₀ 7.63 was determined. Thus, this group is only to some degree protected from viral infection.

5. CONCLUSIONS

[0075] In the present study recovery from body weight loss as well as viral lung titres have been determined to indicate "protection" from a lethal intranasal challenge with VV-WR.

[0076] Control animals challenged with 1x, 12.5x, or 50x the MLD₅₀ of VV-WR revealed a continuous loss of body weight and had a high viral load in the lungs post-mortem (the higher the challenge dose, the higher the viral load detected in the lungs). Thus, these mice were unable to control the infection.

[0077] The smallpox vaccine candidate IMVAMUNE[™] (MVA-BN[®]) was able to protect against an intranasal challenge with up to 50x the MLD₅₀ of VV-WR. This protection was associated with recovery of body weight after initial body weight loss and was also associated with lack of virus in the lungs. The higher the challenge dose of VV-WR, the longer the post challenge period required for body weight recovery in the mice vaccinated 4 days prior to the challenge with MVA-BN[®]: When challenged with 12.5x the MLD₅₀ of VV-WR body weight recovery was detected on day 4 post challenge, whereas body weight recovery was detected on day 5 post challenge when mice have been challenged with 50x the MLD₅₀ of VV-WR. In addition, this study clearly revealed that the time interval between vaccination of mice with MVA-BN[®] and challenge with 1x the MLD₅₀ of VV-WR can be reduced to 2 days, a time interval that is sufficient to enable stabilization of body weight and to obtain reduced lung viral titres following challenge of mice with 1x the MLD₅₀ of VV-WR. Furthermore, increasing the challenge doses

resulted in an extension in the time interval between vaccination with MVA-BN[®] and challenge that is required to obtain protection from the lethal challenge: In case of challenge with 50x the MLD₅₀ of VV-WR a 3 day interval was not sufficient, whereas following challenge with 12.5x the MLD₅₀ of VV-WR a 3 day interval between vaccination and challenge was sufficient to obtain protection.

[0078] In contrast to MVA-BN[®], the first and second generation smallpox vaccines Dryvax and Elstree, respectively, were unable to protect against an intranasal challenge with up to 50x the MLD₅₀ of VV-WR when administered 4 days prior to the challenge. The reason for this difference might be due to the different routes of administration: MVA-BN[®] was administered to mice (and is administered in clinical trials to humans) subcutaneously, whereas Elstree and Dryvax was administered (and was administered to humans) to mice via scarification.

[0079] In summary, this study clearly demonstrates the superiority of MVA-BN[®] over Elstree and Dryvax with regard to onset of protection.

REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

1. Modificeret vacciniavirus Ankara (MVA) til anvendelse i en fremgangsmåde til immunisering af et dyr, herunder et menneske, til beskyttelse mod koppevirusinfektion inden for 2-7 dage efter immunisering, hvor et beskyttende immunrespons mod koppevirus fremkaldes inden for 2-7 dage efter en enkelt vaccination, og hvor MVA er MVA-BN.
2. MVA ifølge krav 1, hvor fremkaldelsen af et beskyttende immunrespons sker inden for 6 dage eller mindre, 5 dage eller mindre, 4 dage eller mindre eller 3 dage eller mindre.
3. MVA ifølge et hvilket som helst af kravene 1 til 2, hvor virusset er et klonoprenset virus.
4. MVA ifølge et hvilket som helst af kravene 1 til 3, hvor virusset er opnået ved en serumfri dyrkningsproces.
5. MVA ifølge et hvilket som helst af kravene 1 til 4, hvor virusset administreres i en dosis på 10^5 til 5×10^8 TCID₅₀/ml.
6. MVA ifølge et hvilket som helst af kravene 1 til 5, hvor virusset administreres intravenøst, intramuskulært eller subkutant.
7. MVA ifølge et hvilket som helst af kravene 1 til 6, hvor virusset administreres subkutant.
8. MVA ifølge et hvilket som helst af kravene 1 til 7, hvor virusset er et rekombinant virus.
9. MVA ifølge krav 8, hvor det rekombinante virus omfatter mindst én heterolog nukleinsyresekvens.
10. MVA ifølge krav 9, hvor den heterologe nukleinsyresekvens er en sekvens, der koder for mindst ét antigen, en antigen epitop og/eller en terapeutisk forbindelse.

11. MVA ifølge krav 10, hvor de antigene epitoper og/eller antigenerne er antigene epitoper og/eller antigener på et smitstof.

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12. MVA ifølge krav 11, hvor det antigene middel er udvalgt blandt vira, svampe, patogene unicellulære eukaryote og/eller prokaryote organismer, parasitiske organismer.

10 13. MVA ifølge krav 12, hvor viraene er udvalgt fra familien af influenzavirus, flavivirus, paramyxovirus, hepatitisvirus, humant immundefektvirus eller blandt vira, der forårsager hæmorrhagisk feber.

15 14. MVA ifølge et hvilket som helst af kravene 1 til 13, hvor fremkaldelsen af et beskyttende immunrespons afspejles ved en serokonversion.

DRAWINGS

Figure 1

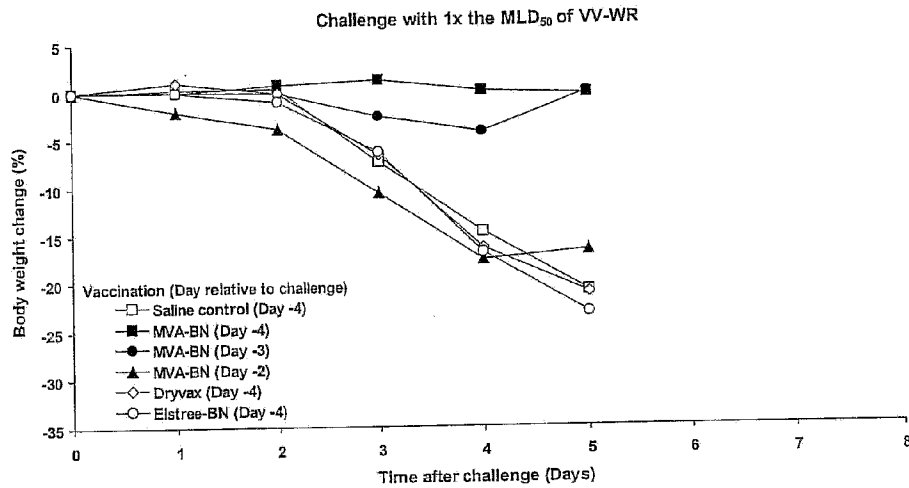


Figure 2

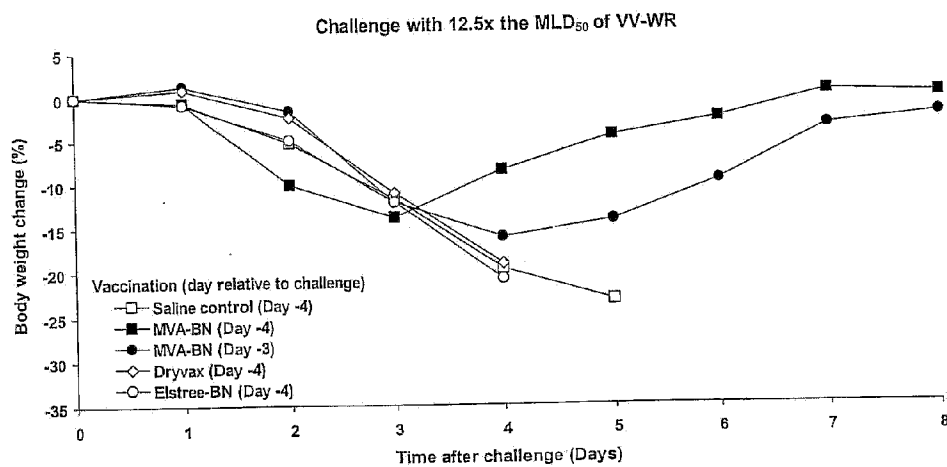


Figure 3

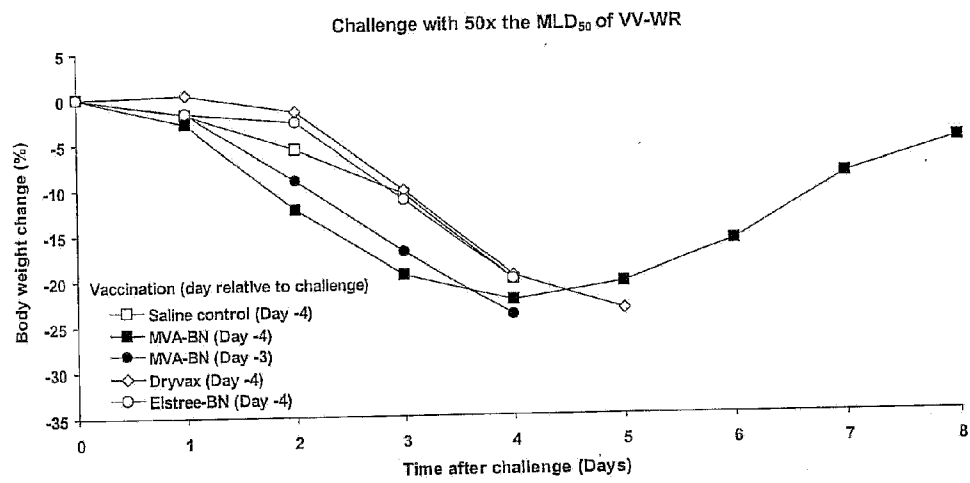


Figure 4

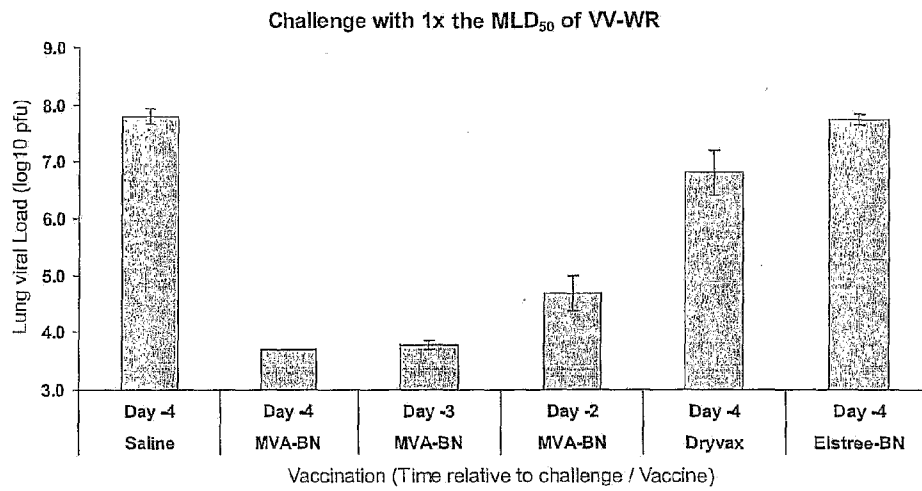


Figure 5

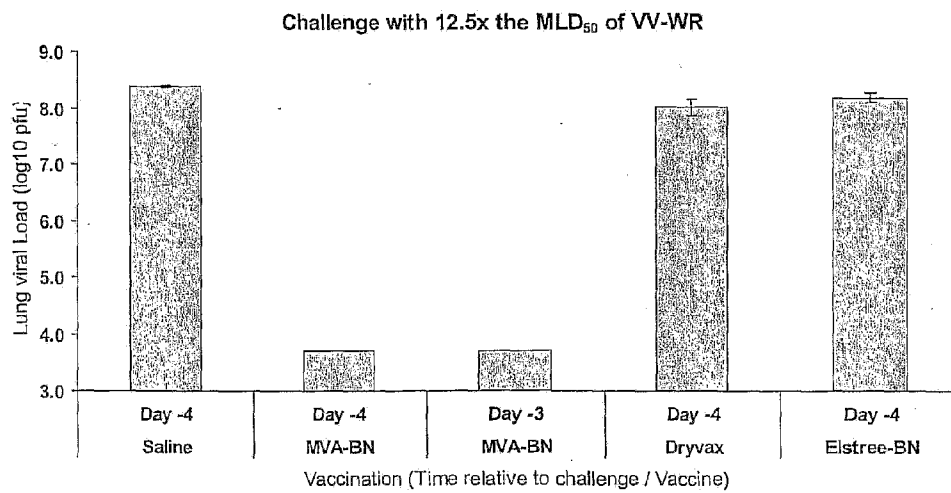


Figure 6:

