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(54) Title: OPERATING PROCEDURE FOR THE PRODUCTION OF VIRUSES AND VIRUS RESISTANT CELLS FOR THE TESTING OF ANTI-VIRAL SUBSTANCES USING LABORATORY ANIMALS

(57) Abstract

Viruses are produced, using laboratory animals that are transplanted with donor cells that can be infected with virus or that are infected by virus. The animals have an immune system that is distorted in such a manner that the transplanted cells are not rejected. The dose of the transplanted donor cells is sufficiently high to ensure an in vivo reaction against the recipient, preferably stimulated by the administration of donor cell growth promoting substances to the laboratory animal. The in vivo reaction against the recipient is preferably an inflammation reaction and more specifically a rejection reaction. In this manner a substantial multiplication of the donor cells is obtained. The combination of multiplication of the donor cells and the multiplication of the virus in these cells results in a very substantial virus-production. The virus production and/or the produced virus are determined and/or quantified by an in vitro virus assay, preferably in vitro tissue culture, ELISA, PCR or immunoblotting. In addition a very sensitive testing system is obtained, which can be used for the testing of anti-viral agents. Finally donor cells can be selected on virus resistance.

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Title: OPERATING PROCEDURE FOR THE PRODUCTION OF VIRUSES AND VIRUS RESISTANT CELLS FOR THE TESTING OF ANTI-VIRAL SUBSTANCES USING LABORATORY ANIMALS.

Field of the invention

The invention relates to the production of viruses, virus-resistant cells and a testing procedure of anti-viral substances directed against the virus.

Background of the invention

Since the *in vitro* production of viruses is very cumbersome and the importance of viral research demands the use of laboratory animals, it has become ethically acceptable to use laboratory animals. The use of these animals however, does have some limitations, more specifically concerning the virus production and the evaluation of anti-viral agents as a result of species-specificity of many viruses.

For this reason three tests for anti viral agents have been developed, each with their characteristic flaws:

The first is the test system of Mc. Cune (Science 247, p.564,1990 and PNAS 88, p4523,1991) contains small pieces of human fetal tissue, implanted in the kidney capsule of so called Severe Combined Immune Deficient(SCID) mice. The draw back of this system is the fact that it is laborious and expensive. In addition, the human immune-system is not yet fully developed, it can not be manipulated, and the total number of donor cells in the mouse remains small.

Another system is the test system of Mosier (Science 251, p 791, 1991). This consists of human peripheral blood cells that are also transplanted in SCID mice, only in a dose that does not result in a rejection towards the mouse. However, in this case only few human cells can be detected afterwards. In addition, these cells do not reveal any immune-function.

The third system is the test system of TNO (Publication number NL 9102122) containing human peripheral blood cells in mice. In this case the dose of the transplanted cells suffices to obtain an acute graft-versus-host disease. Subsequently, it is the objective to cure this disease by adding immune-suppressive viruses. The draw backs here are (a) the only detection is the cure of the disease, (b) in 80 % of the described cases no cure was found at all, (c)no 'curing' viruses have been detected and (d)other detection systems are more sensitive and quantifiable.

Description:

The invention relates to a method in which cells, preferably of the humane immune-system are transplanted, resulting in a reaction towards the laboratory animal. This can be achieved either with or without growth stimulatory agents. Subsequently, in a period of a few days to a few months, whichever is preferred, the reaction results in vast amounts of these cells in the laboratory animal. These cells can be either in vivo or in vitro infected by the virus. In this way vast amounts of virus can be produced.

Donor cells are easily obtained. The starting material can be either blood cells, spleen cells, liver cells, lymph node cells, thymus cells, bone-marrow cells or cancer cells of a solid tumor or a tumor that grows in free cells, including leukemia or other donor cells that are able to perform an in vivo reaction with the host or a comparable cell that is transformed with an agent like a virus.

Examples:

Example 1

The production of virus is performed in a simple procedure. For each animal quantitative results can be obtained about the production of the virus and of the produced virus itself. This is in contrary to the existing alternatives.

The procedure is characterized by the use of laboratory animals with an immune-system that has been disrupted in such a manner, that they do not reject the donor cells after transplantation. In addition, these donor cells are transplanted in a dose which is high enough to initiate an in vivo interaction with the laboratory animal. This in vivo reaction against the recipient has the preference to be an inflammation reaction (as described in European Journal of Immunology 22, p1421, 1992 and in Blood 12, p3440, 1993) and more specifically a rejection reaction (as described in European Journal of immunology 22 p 197 , 1992). In this way a substantial multiplication is caused. The administration of growth promoting agents is able to stimulate the multiplication of the transplanted donor cells.

Subsequently, it is preferred to use small laboratory animals like rabbits, hamsters, guinea pigs, rats, mice and more specifically mice with the SCID or X-linked Immune-deficient (XID) mutation.

Furthermore it has the preference to pre-treat the laboratory animal with for instance gamma-, X-ray or neutron-irradiation, cytostatics or antibodies against lymphocytes with the purpose to distort the immune system of the animal. A very suitable method has proven to be the treatment of XID mice with 8 to 10 Gy gamma irradiation. An other suitable method has proven to be the treatment of SCID mice with 2-4 Gy gamma irradiation.

Furthermore, treatment of the animal with the distorted immune system is found to be preferable, in preference with autologous bone marrow and in preference in a dose of 5000 to 0.5 million cells per gram of laboratory animal.

Also, it is preferred that human lymphocytes, lymphoblasts and/or macrophages are administered, preferably in a dose of at least 40 000 cells per gram of laboratory animal. It is also found to be preferable to add growth promoting substances to stimulate cellular multiplication of the donor cells. These growth promoting substances were preferably mitogens or proteins, preferably interleukins, interferons or tumor necrosis factor. The virus was preferably administered as primary isolate of an infected human, or as culture fluid after secretion by cells in a culture-flask or as infected cells. The preferred research is about human viruses, in preference the human immune deficiency (HIV) virus.

In principle, any in vitro read out system could be used , for instance cell culture ,PCR, electrophoresis, hybridization, fluorescent cytometry, ELISA. However, it was our preference to quantify the virus content in the laboratory animal by a titration on a cell line which is sensitive to this virus, by preference HIV was cultured on a $CD4^+$ human cell .

Example 2

The invention also relates to laboratory animals with virus producing donor cells, characterized by the in vivo reaction of these cells with the laboratory animal. Preferably this reaction can be stimulated by the addition of donor cell growth promoting substances. This in vivo reaction against the laboratory animal has the preference to be an inflammation reaction and more specifically a rejection reaction against the laboratory animal.

This procedure leads to the selection of virus, for instance on tropism, virulence or on expression of gene-products as intended in gene therapy. Therefore, all viruses produced in this manner are also regarded to be within the scope of this invention.

Example 3

An application of the invention is also a method to test anti viral agents directed against the virus. In this case the inhibition of virus production is determined. Both treatments that prevent the spread of the virus and agents that inactivate the virus can be tested. It can be stated that the efficiency of our production system enables a high sensitivity of the test.

Example 4

An other application of the invention is the selection of donor cells that have become virus resistant, preferably by genetic manipulation. Therefore, also the thus obtained virus resistant cells are also regarded to be within the scope of the invention.

What is claimed is:

1. A procedure for the production of virus including the use of laboratory animals which were transplanted with donor cells , that can be infected by virus or donor cells that are infected by virus, wherein an in vivo reaction of the donor type cells occurs against the laboratory animal .

2. Procedure according to claim 1, wherein the in vivo reaction towards the animal is an inflammation reaction.

3. Procedure according to claim 1 and/or 2, wherein the in vivo reaction towards the animal is a rejection reaction.

4. Procedure according to one or more of the preceding claims wherein a substantial multiplication of the donor cells occurs.

5. Procedure according to one or more of the preceding claims, wherein growth promoting substances are used to stimulate multiplication of donor cells.

6. Procedure according to one or more of the preceding claims, wherein small laboratory animals are used, for instance mice, rats, hamsters guinea pigs and rabbits.

7- Procedure according to one or more of the preceding claims, wherein mice with a SCID or XID mutation are used.

8- Procedure according to one or more of the preceding claims, wherein rejection of donor cells is prevented by the use of animals with a distorted immune system.

9- Procedure according to one or more of the preceding claims, wherein the distortion of the immune-system of the animal is performed by a high dose of total body irradiation. (gamma-, X-ray or neutron irradiation) and/or a high dose of cytostatics or antibodies against lymphocytes.

10- Procedure according to one or more of the preceding claims, wherein the animal is treated with gamma irradiation , preferably XID-mice with a dose of 8-10 Gy or SCID mice with a dose of 2-4 Gy.

11- Procedure according to one or more of the preceding claims, wherein the animal is treated with bone-marrow, preferably autologous bone-marrow, preferably in a dose of 5000 to 0.5 million cells per gram of laboratory animal.

12- Procedure according to one or more of the preceding claims, wherein the administered donor cells are primate cells, by preference human cells.

13- Procedure according to one or more of the preceding claims, wherein the administered donor cells are human lymphocytes.

14- Procedure according to one or more of the preceding claims, wherein the administered donor cells are human lymphoblasts.

15- Procedure according to one or more of the preceding claims, wherein the administered donor cells are human macrophages.

16- Procedure according to one or more of the preceding claims, wherein the human donor cells are administered in a dose of at least 40 000 per gram of laboratory animal.

17. A procedure according to one or more of the preceding claims, wherein the administered virus is a human virus.

18. A procedure according to one or more of the preceding claims, wherein the administered human virus is a human immuno deficiency virus (HIV) .

19. A procedure according to one or more of the preceding claims, wherein it is tissue is isolated from the animal to obtain the produced virus preferably to quantify the virus in an in vitro manner.

20. Laboratory animals, obtained as described in one or more of the preceding claims.

21. Viruses, obtained as described in one or more of the claims 1-19.

22. A procedure to obtain virus resistant donor cells, as described in one or more of the claims 1-18.

23 Donor cells, obtained as described in one or more of the preceding claims.

24 A procedure for the testing of anti viral substances, further characterized by the determination of the inhibition of virus production.