25 FEB 2004

NOVEL USE OF ANTIBODIES AS VACCINES

The present invention describes the use of antibodies, which bind to antibodies directed against tumour-associated antigens and are recovered from individual body fluids containing antibodies by immunoaffinity purification on specific ligands, for the production of a composition for the individual, autologous prophylactic or therapeutic vaccination against cancer diseases. The ligands of the immunoaffinity purification are antibodies or derivatives thereof which are directed against one or more tumour-associated antigens. Furthermore, the invention relates to pharmaceutical compositions containing the antibodies obtained by the immunoaffinity purification or dendritic cells pulsed therewith in vitro.

The adaptive immune system of humans consists of two essential components, i.e. the humoral and the cellular immunity. The adaptive immune response is based on the clonal selection of B and T lymphocytes and, in principle, allows for any antigen to be recognised and an immunologic memory to be formed. In the case of vaccinations, advantage is generally taken of these features of the adaptive immune system.

Every B cell produces an antibody with a certain binding specificity. This antibody is also present as a specific receptor in the membrane of those B cell by which it is produced. The humoral immune response against antigens which are recognised as foreign antigens is based on the selective activation of the B cells which produce antibodies that are capable of binding to epitopes of the respective antigen. In the course of the differentiation of the B cells, DNA rearrangements are crucial as regards the antibody diversity.

In human serum, there is a large number of antibodies of the most varied specificities, isotypes and subclasses. The overall concentration of all immunoglobulins in the serum is 15 to 20 mg/ml; i.e. about 100 g of immunoglobulins having the most varied specificities circulate permanently in the blood. It is not

25 FEB 2004

possible to indicate the exact amount of all antibodies having different specificities. The repertoire which is theoretically possible is about 10^{11}. In general, a certain antibody may bind different antigens which are similar to each other, although with a different affinity and avidity.

The immune system has to maintain a homoeostasis as regards the distribution and importance of these different specificities by means of endogenous regulation mechanisms. An essential mechanism is the "idiotypic network" which was postulated by Niels Jerne about 25 years ago (Jerne, Ann. Immunol. 125C (1974), 373-389): There are anti-idiotypic antibodies which are directed against every idiotype of an antibody determining its binding specificity and which, therefore, bind to the idiotype of the first antibody within the meaning of an antigen recognition. Jerne suggested that the interactions between the idiotype-specific receptors on lymphocytes may be responsible for the regulation of the immune system. These interactions obviously take place indeed since it has been shown that, in the course of an immune response, also anti-idiotypic antibodies directed against the antibodies primarily induced by the immune response are formed. As there are anti-idiotypic antibodies against every antibody, lymphocytes are principally not tolerant as regards idiotypes of antibodies.

Within an immunologic meaning, some anti-idiotypic antibodies can represent the "internal image" of an antigen. Such antibodies may therefore be used for immunisation as a surrogate of the nominal antigen since the antibodies induced by an immunisation can, in part, bind to the nominal antigen. For quite a long time, this approach has been used, in particular in cancer immunotherapy. For more than 10 years, there is a number of pre-clinical and clinical projects for inducing immune responses against tumour-associated antigens by vaccination with monoclonal anti-idiotypic antibodies (for a summary see: Bhattacharya-Chatterjee, Foon; Anti-idiotype antibody vaccine therapies of cancer; Cancer Treat. Res. 94 (1998), 51-68). Most of these monoclonal anti-idiotypic antibodies are of murine origin. There are, however, also tests with human monoclonal anti-idiotypic antibodies (e.g. Fagerberg et al., PNAS 92 (1995), 4773-4777).

Attempts for the therapeutic immunisation of patients suffering from B cell lymphomas are a particular case. In the case of such a disease, a certain B cell clone which produces a specific immunoglobulin having an idiotype characteristic thereof and which also carries said immunoglobulin as a receptor in the cell membrane is degenerate. This antibody is monoclonal and can be regarded as a tumour-specific antigen for the individual B cell lymphoma. It has been shown that such patient-specific autologous antibodies, when produced in cell culture and administered as a vaccine in a suitable immunogenic form, can induce an immune response against the idiotype of this antibody, said immune response being able to have an effect on the B

25 FEB 2004

ANNEX IPER

(amended pages 3 and 3a of the specification)

cell lymphoma (Reichardt et al., Blood. 93 (1999), 2411-2419).
To sum up, it can be said that

- human monoclonal anti-idiotypic antibodies which mimic a tumour-associated antigen can, as a vaccine, induce an immune response in cancer patients, said immune response being possibly directed against said tumour-associated antigen. By means of hybridoma technology or immortalisation of human B cells, such human monoclonal antibodies have been obtained from patients which had been given a (mostly murine) anti-tumour antibody for passive immunotherapy and which had established an immune response thereagainst. Other patients were immunised with human monoclonal anti-idiotypic antibodies produced in this manner.
- human antibodies which are produced by a B cell lymphoma and therefore have a defined idiotype can, as an individual vaccination, induce an immune response in a patient-specific manner, said immune response possibly being directed against the B cell lymphoma. Such patient-specific antibodies must be obtained individually by means of hybridoma technology or immortalisation of the cells of the B cell lymphoma. The use of said antibodies is naturally limited to the treatment of B cell lymphomas.

Therefore, although it has been known that monoclonal anti-idiotypic antibodies from a certain species can also be used for immunising individuals of the same species from which the antibodies are derived, it is in any case necessary to manipulate and cultivate cells. Thus, this approach is time-consuming and is limited to monoclonal antibodies only.

In Losman et al. Proc. Natl. Acad. Sci. USA 88 (1991), 3421-3425, the purification of ab2 antibodies from a cancer patient has been described. Yet, this patient was treated before with a murine monoclonal antibody (NP-4) against CEA.

Hence, it is clear that there is still a need for providing means and methods which allow an effective and broadly applicable and, optionally, individual treatment of tumours as well as a protection against cancer.

Therefore, the technical problem underlying the present invention is to provide efficient means and methods, which can be used individually within the framework of

25 FEB 2004

different kinds of tumours, for the treatment of cancer and for the protection against cancer.

This technical problem has been solved by providing the embodiments characterised in the claims.

Thus, the present invention relates to the use of antibodies for the production of a composition which is suitable as a vaccine for the therapeutic of prophylactic

25 FEB 2004

vaccination against cancer characterised in that the antibodies are recovered from body fluids containing antibodies by immunoaffinity purification, wherein antibodies recognising one or more tumour-associated antigens or the fragments thereof having the same idiotype are used as ligands for the immunoaffinity purification.

In comparison with the methods described in the state of the art, the use according to the invention, has the advantage that no cell cultures are used for producing the anti-idiotypic antibodies which makes it possible that the concept of the invention is applied broadly and effectively and is not limited to monoclonal antibodies.
The pool of antibodies which are, for instance, present in a large overall amount in the blood of every human contains antibodies of every possible binding specificity. Thus, this antibody pool, in principle, also contains antibodies (Ab2) against the idiotype of e.g. (already known or also new) murine monoclonal antibodies (Ab1) directed against any tumour-associated antigen (TAA). In accordance with the immunologic network, the same antibody pool in every human also contains a certain amount of antibodies directed against the idiotypes of the autologous Ab2, the so-called Ab3 antibodies. According to network theory, Ab3 can bind to the tumour-associated antigen which is defined by the external Ab1. Shifting the equilibrium of Ab2/Ab3 in favour of Ab3 must result in the immune system being able to better recognise and attack tumour cells carrying said antigen.
It is now the basis of the present invention that such shifting of the immunologic equilibrium can be achieved by administering an individual autologous Ab2 fraction in form of an immunogenic vaccine. By such an autologous vaccination, those B cells which produce Ab3 can be stimulated selectively. For such a vaccination, only small amounts of the Ab2 fraction are necessary which can be formulated according to known methods using a suitable vaccine adjuvant.
The antibodies used according to the invention are therefore Ab2 antibodies from body fluids containing antibodies and which are directed against the anti-TAA antibody used for the immunoaffinity purification. Body fluids containing antibodies are, for instance, blood, plasma, serum, lymph, malign effusion, etc. The body fluid is preferred to be serum. The body fluid can be derived from any animal organism which has body fluids containing antibodies. Preferably, these are vertebrates, more preferably mammals and most preferably, the body fluid is of human origin.
As the immune response induced by the vaccination with autologous antibodies is determined by the binding region of these antibodies, i.e. by their idiotype, in principle, also fragments or derivatives of these antibodies can be used for successful vaccination instead of intact antibody fractions as long as these fragments or derivatives still contain the idiotype of the respective starting antibody. Thus, the term "antibody" also comprises fragments or derivatives of such antibodies having

the same binding specificity. As examples, but with no limitation thereto, the following are mentioned: $F(ab)'_2$ fragments and F(ab)' fragments which can be produced, for instance, according to biochemical methods known per se (e.g. by enzymatic cleavage). The term "derivative" comprises e.g. antibody derivatives which can be produced according to chemical or biochemical methods known per se, such as, e.g. antibodies amidated with fatty acids on free amino functions for increasing the lipophilia for incorporation into liposomes. In particular, the term "derivative" also comprises products which can be produced by chemically coupling antibodies or antibody fragments with molecules which can enhance the immune response, such as, e.g. tetanus toxoid, Pseudomonas exotoxin, derivatives of lipid A, GM-CSF, IL-2 or by chemically coupling antibodies or antibody fragments with polypeptides which enhance the immune response, such as, e.g. GM-CSF, IL-2, IL-12, C3d, etc. The purification of the antibodies from body fluids containing antibodies, e.g. from human serum, by means of immunoaffinity purification can be carried out using methods known to the person skilled in the art (Clin. Chem. 45 (1999), 593; J. Chem. Technol. Biotechnol. 48 (1990), 105). A TAA-specific antibody or a mixture of antibodies is immobilised on a solid phase. The solid phase can be a membrane, a gel or a similar material which antibodies can be bound to without an essential loss in the binding properties. The Ab2 can be bound to the immobilised antibodies in batches or in a flow-through method. The immunoaffinity purification can be carried out automatically on a chromatography device or by means of a manual method. It is, however, also conceivable that the method is carried out manually, automatically or semi-automatically by means of a simple device containing the immobilised antibodies. In an immunoaffinity chromatography, which is described in Example 1, usually between 3 to 10 µg immunoglobulin per ml serum used are obtained. In general, the antibody fraction obtained in this way consists of both IgM and IgG. It is therefore possible to recover about 0.08 to 0.25 mg Ab2 from an amount which is acceptable for blood taking, e.g. 50 ml leading to about 25 ml serum. This amount is basically sufficient for vaccination. If larger amounts of Ab2 are wished to be recovered, techniques such as plasmapheresis, which are known per se, can be used in combination with immunoaffinity purification. If several antibodies having different specificities are used, these immobilised antibodies can be used simultaneously or separate immunoaffinity purifications can be carried out in parallel or in series.

Within the framework of the present application, the term "tumour-associated antigen" is a structure which is preferably presented by tumour cells and which makes it therefore possible to differentiate it from non-malignant tissue. Preferably, such a tumour-associated antigen is localised on or in the cell membrane of a tumour cell. It is not ruled out, however, that such antigens are also present on non-degenerate cells. Tumour-associated antigens may be, for example, polypeptides, in

particular glycosylated proteins, or glycosylation patterns of polypeptides. Other structures which may represent a tumour-associated antigen are, for instance, glycolipids. These include, for example, gangliosides, such as, e.g. GM2. Furthermore, tumour-associated antigens can be represented by changes in the composition of lipids of the cell membrane which may be characteristic of cancer cells.
The most varied antibodies against different tumour-associated antigens have been known for a long time or can be produced using methods known per se (e.g. hybridoma technology, phage display technology).

In a preferred embodiment, the antibodies obtained by the immunoaffinity purification are administered in the sense of an autologous, individual vaccine, to the individual from whose body fluid they were recovered.
Therefore, it is a further integral component of the present invention that the recovery of an Ab2 fraction from the body fluid of an individual, e.g. blood, can be achieved by means of immunoaffinity purification of the antibodies present in the body fluid. One – or more – antibody/antibodies against tumour-associated antigens is/are used as a ligand.
In this context, an individual may be any animal organism, preferably a vertebrate, more preferably a mammal and most preferably a human.

In another preferred embodiment, several antibodies directed against various tumour-associated antigens and/or against various epitopes of one or more tumour-associated antigens can be used at the same time for the immunoaffinity purification. If several, in particular monoclonal, antibodies directed against different tumour-associated antigens or against different epitopes are used at the same time for the immunoaffinity purification of the Ab2 present in the body fluid, a fraction consisting of different Ab2 is obtained which is based on the selected set of tumour-associated antigens and the simultaneous use of which as a vaccine triggers an immune response against all these tumour-associated antigens or epitopes. Thus, the immunologic equilibrium is shifted in one step towards the recognition of a set of (often co-expressed) tumour-associated antigens or epitopes. Such a procedure naturally increases the effectiveness of the immune response induced and essentially reduces the formation of antigen-negative variants of the tumour ("tumour escape") since it is extremely unlikely that a tumour cell can stop the expression of several antigens at the same time.
In principle, the method described above can be based on all known or newly discovered tumour-associated antigens. The only precondition is that one or more antibodies, preferably monoclonal antibodies, directed against such an antigen are

25 FEB 2004

available which can be used as a ligand for an immunoaffinity purification. In principle, all possible kinds of antibodies, in particular polyclonal or monoclonal antibodies, are suitable, with monoclonal antibodies being preferred. It is also possible to use mixtures of polyclonal and monoclonal antibodies. Furthermore, both, e.g. murine monoclonal antibodies and antibodies from other species (e.g. rats) can be used. Moreover, mouse-human chimeric, humanised or human monoclonal antibodies against tumour-associated antigens can be used. The property of the antibodies used for the immunoaffinity purification is determined by their binding region, i.e. their idiotype. Thus, in principle, also fragments of these antibodies can be used for a successful immunoaffinity purification instead of intact antibodies as long as these fragments still keep the idiotype of the respective starting antibody. Examples, however without limitation thereto, include: $F(ab)'_2$ fragments, F(ab)' fragments and Fv fragments which can be produced according to biochemical methods known per se (enzymatic cleavage) or according to methods of molecular biology known per se. As a matter of fact, it is also possible to use mixtures of the respective kinds of antibodies or fragments.

The selection of the tumour-associated antigens and therefore of the antibodies used for the immunoaffinity purification, which are directed against these antigens, depends on the antigen characteristics of the tumour indication against which a vaccination is to be carried out.

The following tumour-associated antigens which have been known already and which are frequently expressed on various, in particular, epithelial tumours are listed exemplarily. Yet, the use of the method of autologous vaccination, which is described herein, is not limited to these antigens at all:

epithelial cell adhesion molecule (Ep-CAM)
carcino embryonic antigen (CEA)
Lewis Y carbohydrate
sialyl Tn carbohydrate
globo H carbohydrate
gangliosides such as GD2 / GD3 / GM2
prostate specific antigen (PSA)
CA 125
CA 19-9
CA 15-3
TAG-72
EGF receptor
neuronal cell adhesion molecule (N-CAM)
Her2/Neu receptor

25 FEB 2004

D 97
CD 20
CD 21

Against all the antigens mentioned (mostly several), in particular monoclonal antibodies have been described which are principally suitable for being used as ligands for the aforementioned immunoaffinity purification for recovering Ab2.
Further tumour-associated antigens are described, for instance, in DeVita et al. (Eds., "Biological Therapy of Cancer", 2nd edition, chapter 3: Biology of Tumour Antigens, Lippincott Company, ISBN 0-397-5144116-6 (1995)).

In a preferred embodiment, the tumour-associated antigen is a membrane-located molecule which is frequently expressed or co-expressed on cancer cells of epithelial origin, on cancer cells of neuroendocrine origin or on cancer cells of the blood-forming system.

In another preferred embodiment, the composition produced according to the invention is suitable for a multiple administration of the antibodies contained therein. The method described herein for recovering an autologous vaccine from the body fluids of an individual naturally is not limited to one single application.
The shifting of the immunologic repertoire towards anti-tumour effectiveness, which is triggered by the first vaccination, can be enhanced by repeating said procedure, e.g. a few weeks after recovering the first autologous vaccines by immunoaffinity purification, body fluid, e.g. blood can be taken again and a new autologous vaccine can be prepared and administered. In this way, it is guaranteed that the corresponding status of the immunologic equilibrium in the individual vaccine is always taken into account. This process can be carried out again and again in suitable intervals (e.g. at first every 4 to 8 weeks, then every 6 months).

The new composition and method for vaccination with autologous antibodies, which are described herein, are in principle suitable for both therapeutic and prophylactic purposes. A repeated therapeutic vaccination of cancer patients can suppress the formation of new metastases and can at least slow down the dissemination of the disease. Therapeutic autologous vaccinations can be useful in particular in the following stages of the disease:
In early stages of the disease, e.g. after a successful operation of a primary tumour (adjuvant stage), remaining, disseminated tumour cells are destroyed by the autologous vaccinations described herein and are prevented from forming new metastases. As a consequence, the relapse-free lifespan and thus the overall

25 FEB 2004

survival time of such patients can be prolonged. Optionally, it is possible to achieve a lifelong protection from the formation of metastases by means of such autologous vaccinations and booster shots administered in suitable intervals.
If metastases have already been formed, the spreading and formation of further metastases can be contained by means of the composition or the autologous vaccinations described herein. The status of the disease is stabilised and, as a result, the quality of life can be maintained and the lifetime can be extended.
The strategy of autologous vaccinations described herein can also be used in patients to whom has been administered a monoclonal antibody against a tumour-associated antigen before for reasons of diagnosis or therapy and who have developed an immune response thereagainst. As a consequence, in an immunoaffinity purification in which this antibody is used as a ligand, antibodies directed thereagainst (Ab2) are obtained in an amount larger than in the case of non-treated individuals. The vaccination with these Ab2 enhances the formation of Ab3 which have possibly already been formed intrinsically. The intrinsic induction of Ab3 with potential anti-tumour properties via induction of Ab2 by means of a passive immunotherapy with murine antibodies directed against tumour-associated antigens (Ab1), which are administered to cancer patients i.v., has been postulated and analysed for many years (e.g. Koprowski et al., PNAS 81 (1984), 216-19).
The composition and autologous vaccinations described herein can, in principle, also be used for increasing, as a prophylaxis, the protection against the formation of cancer diseases in healthy individuals. Such a measure can make sense, in particular in the case of high-risk groups (these include e.g. individuals with a genetic disposition to develop certain cancer diseases, which can be detected increasingly by means of corresponding tests).
The fact that the immunologic status of the respective individual as regards the idiotypic network is taken into consideration since the corresponding vaccine is preferred to be produced from the individual body fluid, e.g. serum, is a general advantage of the strategy of individual autologous vaccination described herein.
Furthermore, as a consequence, the immunised individual does not get in contact with foreign antigens but is treated only with endogenous components in a suitable form which effect the modulation of the immunologic equilibrium.

In a preferred embodiment according to the invention, the antibody obtained by immunoaffinity purification is formulated with a suitable vaccine adjuvant.
As is common with vaccines, the autologous antibody fractions or their fragments and derivatives can be formulated together with vaccine adjuvants. The immune response is enhanced by such adjuvants. Examples of such adjuvants, however without limitation thereto, include: aluminium-containing adjuvants, in particular aluminium

25 FEB 2004

hydroxide (e.g. Alu-gel), derivatives of lipopolysaccharide, Bacillus Calmette Guerin (BCG), QS-21, liposome preparations, formulations with additional antigens against which the immune system has already developed a strong immune response, such as, e.g. tetanus toxoid or components of influenza viruses, optionally in a liposome preparation.

For enhancing the immune response, the vaccine preparation can also be administered together with corresponding, preferably human, cytokines which support the formation of an immune response. In particular, however not exclusively, the granulocyte-macrophage stimulating factor (GM-CSF) is to be mentioned. This cytokine stimulates an effective immune response by the activation of antigen-processing cells (e.g. dendritic cells).

Optionally, by using methods which are known per se and which have been published, the autologous antibody fractions can also be incubated with autologous dendritic cells cultivated ex-vivo. The dendritic cells pulsed in this way are subsequently administered to the respective individual. It is possible to achieve a particularly effective immune response in this way.

In a preferred embodiment of the use according to the invention, the antibodies contained in the composition are mixed with an adjuvant and are then subjected to a heat treatment, preferably at a temperature between 70 and 121°C. The adjuvant used is preferably an aluminium-containing adjuvant. It is possible that, although such a heat treatment denatures the protein antigen, the immunogenic parts of the protein can be presented to the immune system in the correct form due to the binding to the adjuvant. Yet, it is not absolutely necessary to denature the proteins in order to achieve the advantages of a heat treatment. It is known that the thermal denaturation of proteins does not only depend on the temperature, but also on the time for which the protein is subjected to this temperature. Moreover, there are other physicochemical parameters, such as, e.g. ion strength, composition of the ions, pH value, kind and amount of the active surface in the mixture, which are responsible for the denaturation of a protein. There may be conditions under which the antibodies are not or not entirely denatured and/or other effects, such as, e.g. less desorption on the surface of the adjuvant can be used.

A further advantage of such a way of producing a vaccine formulation with an adjuvant and a subsequent heat treatment is that infectious pathogens could be weakened or inactivated in the entire formulation. This advantage can be of importance for both the production and the storage and distribution of the vaccine formulation. Thus, there is greater safety with regard to known and unknown pathogens of transmittable diseases. Moreover, it is possible to fill it up without preservatives if a corresponding packing is used since the microbial preservation of

25 FEB 2004

the vaccine has been carried out by means of heat.
Another advantage of such a formulation is the potentially increased immunogenicity of the antibodies as the heating can lead, at least in part, to the denaturation of the antibodies. This increased antigenicity can increase the immunogenicity, in particular in the case of proteins that would be recognised as the body's own proteins.
Another advantage is that the antibody adjuvant complex is potentially additionally stabilised by the heat inactivation, i.e. the desorption of the protein antigen does not take place as fast as in antigen adjuvant formulations which have not been treated with heat. This advantage possibly also permits a larger interval between the individual immunisations.

The composition produced according to the invention can be administered in accordance with commonly known methods, e.g. as a vaccine by subcutaneous or intramuscular injection.

Furthermore, the present invention relates to pharmaceutical compositions containing antibodies which have been recovered from body fluids containing antibodies by means of immunoaffinity purification, wherein antibodies recognising one or more tumour-associated antigens or fragments thereof having the same idiotype are used as ligands for the immunoaffinity purification.
As described above, such compositions are suitable as vaccines for the therapeutic or prophylactic vaccination against cancer.
With respect to the preferred embodiments of the preparation, the components, the formulation, the kinds of administration, etc. of the pharmaceutical composition the same applies as has been explained earlier in connection with the use according to the invention.

Last but not least, the present invention also relates to a pharmaceutical composition containing isolated dendritic cells of an individual which have been cultivated ex vivo, wherein the dendritic cells have been incubated in vivo with antibodies which had been recovered from an antibody-containing body fluid of the same individual by means of immunoaffinity purification.
In addition, the present invention also relates to methods for the therapeutic or prophylactic vaccination against cancer, wherein an individual is administered antibodies which have been obtained from a body fluid containing antibodies, preferably from a body fluid of the same individual, by means of immunoaffinity purification, wherein antibodies recognising one or more tumour-associated antigens or fragments thereof having the same idiotype are used as ligands for the immunoaffinity purification.

Moreover, the present invention relates to a method for the therapeutic or prophylactic vaccination against cancer, wherein an individual is administered autologous, isolated dendritic cells which have been cultivated ex vivo and which have been incubated before in vitro with antibodies which have been obtained from body fluids containing antibodies by means of immunoaffinity purification, wherein antibodies recognising one or more tumour-associated antigens or fragments thereof having the same idiotype are used as ligands for the immunoaffinity purification.
The same as has been explained earlier in connection with the use according to the invention applies to the preferred embodiments.

The present invention also relates to a method for the production of an antibody preparation characterised in that the antibodies are recovered from a body fluid containing antibodies by immunoaffinity purification, wherein antibodies recognising one or more tumour-associated antigens or fragments thereof having the same idiotype are used as ligands for the immunoaffinity purification.
The same as has been explained earlier in connection with the use according to the invention applies to the body fluids, the antibodies used as ligands as well as to the tumour-associated antigens.
The immunoaffinity purification can be carried out according to methods known to the person skilled in the art. In this case, too, the same as has been mentioned earlier in connection with the use according to the invention applies.

In a preferred embodiment of the method of the invention, several antibodies directed against different tumour-associated antigens and/or epitopes of such antigens are used at the same time for the affinity purification. In the approach in which several antibodies are used for the affinity purification, they can be used either simultaneously or separate immunoaffinity purifications can be carried out in parallel or in series, i.e. different epitope and/or antigen specificities can be combined as required. These requirements result from the expression of different antigens on different individual tumours.

In a particularly preferred embodiment, monoclonal antibodies are used as ligands in the immunoaffinity purification and in an even more particularly preferred embodiment serum is used as a body fluid.

The method of the invention preferably is a method for the preparation of an antibody preparation which is used for the production of a pharmaceutical composition that is suitable for the therapeutic or prophylactic vaccination against cancer.

25 FEB 2004

Furthermore, the present invention relates to a method for the preparation of a pharmaceutical composition, wherein an aforementioned method of the invention for the preparation of an antibody preparation is carried out and the antibody preparation obtained in this way is subsequently formulated with a pharmaceutically acceptable carrier and/or a suitable vaccine adjuvant.

In a preferred embodiment, the antibody preparation is mixed with an adjuvant, preferably with an aluminium-containing adjuvant, and is then subjected to a heat treatment, preferably at a temperature between 70 and 121°C.

The same as has been mentioned earlier in connection with the use according to the invention applies to the preferred embodiments of such a pharmaceutical or vaccine formulation.

The disclosure content of all of the patents, publications or databank entries cited in this application is herewith in its entirety incorporated into the disclosure content of the present application by reference.

Figure 1 shows a chromatogram of an immunoaffinity purification (by means of the immobilised antibody 17-1A) of the antibody fraction from the serum of a rhesus monkey.

Figure 2 shows a chromatogram (size exclusion chromatography) of an antibody fraction purified by immunoaffinity purification.

Figure 3 shows the binding of serum antibodies obtained by affinity purification on antibody 17-1A: 17-1A ELISA.

Figure 4 shows the result of an autologous vaccination of a rhesus monkey: binding of serum-Ig to KatoIII tumour cells (cell ELISA).

Figure 5 shows a chromatogram (size exclusion chromatography) of an antibody fraction purified by immunoaffinity purification (mixed-bed anti-EpCAM/anti-LewisY).

Figure 6 shows a chromatogram of a size standard (separated by size exclusion chromatography).

25 FEB 2004

The following materials were used in the Examples below which are to illustrate the present invention in more detail but which are not to limit it:

microtiter plates:	Immuno Plate F96 MaxiSorp (Nunc) for ELISA Cell Culture Cluster (Costar; Cat. no. 3598) for cell ELISA
cell line:	KATO III: human stomach cancer cell line (ATCC HTB 103)
coupling buffer:	0.1 M $NaHCO_3$ 0.5 M NaCl pH 8.0
purification buffer A:	PBS def 0.2 M NaCl
purification buffer B:	0.1 M glycine/HCl 0.2 M NaCl pH 2.9
medium A:	RPMI 1640 + 2 g/l $NaHCO_3$ 100 U/ml penicillin G 100 µg/ml streptomycin sulphate 4 mM glutamine 10% foetal calf serum (heat-inactivated)
binding buffer:	15 mM Na_2CO_3 35 mM $NaHCO_3$ 3 mM NaN_3 pH: 9.6
PBS deficient (def):	138 mM NaCl 1.5 mM KH_2PO_4 2.7 mM KCl 6.5 mM Na_2HPO_4 pH: 7.2
fixing solution:	0.1% glutardialdehyde in physiological saline solution

25 FEB 2004

washing buffer A:	2% NaCl 0.2% Triton X-100 in PBS deficient
washing buffer B:	0.05% Tween 20 in PBS deficient
blocking buffer A:	5% foetal calf serum (heat-inactivated) in PBS deficient
blocking buffer B:	1% bovine serum albumin 0.1% NaN_3 in PBS deficient
dilution buffer A:	2% foetal calf serum (heat-inactivated) in PBS deficient
dilution buffer B:	PBS deficient
staining buffer:	24.3 mM citric acid 51.4 mM Na_2HPO_4 pH: 5.0
substrate:	40 mg o-phenylene diamine dihydrochloride 100 ml staining buffer 20 µl H_2O_2 (30%)
stop solution:	4 N H_2SO_4
formulation buffer:	10% PBS def, pH = 6.0 90% physiological saline solution

Example 1

Preparation of an immunoaffinity column with a TAA-specific antibody

7.5 g CH-sepharose 4B (Pharmacia) were suspended for 15 minutes in 20 ml 1 mM HCl. The gel was then washed with 1 l 1 mM HCl and subsequently with 200 ml coupling buffer on an AG3 filter of sintered glass. 100 mg murine antibody 17-1A (Panorex, stock solution 10 mg/ml; directed against the tumour-associated antigen Ep-CAM) were dialysed against 5 l coupling buffer and adjusted to 5 mg/ml with

25 FEB 2004

coupling buffer. This solution was mixed with the gel suspension in a closed container. A suspension which is suitable for coupling can be achieved with a ratio of gel to buffer of 1:2. This suspension was rotated for 24 hours at 4°C. Then, the excess of ligand was washed off with 3 x 30 ml coupling buffer. The remaining reactive groups were blocked by an 1-hour incubation with 1 M ethanol amine at 4°C. Then, the gel was rotated for 1 hour at room temperature with 0.1 M Tris-HCl buffer. Finally, the gel was washed with three cycles of buffers with alternating pH values. Every cycle consists of 0.1 M sodium acetate buffer, pH 4 with 0.5 M NaCl, and subsequently 0.1 M Tris-HCl buffer, pH 8 with 0.5 M NaCl. The gel was stored at 4°C.

Example 2

Immunoaffinity purification of an antibody fraction from serum by means of an immunoaffinity chromatography

10 ml peripheral blood were taken from rhesus monkeys and serum was recovered therefrom. The entire procedure was conducted under sterile conditions.

The immunoaffinity purification was carried out on an FPLC system (Pharmacia). 1 ml of the gel obtained according to Example 1 was filled into an HR5/5 column by Pharmacia. 5 ml serum were diluted 1:10 with the purification buffer A. This solution was pumped over the column with 1 ml/minute and then washed with purification buffer A until the UV base line of the detector was reached again (280 nm). Bound immunoglobulins were eluted with purification buffer B and the fraction was neutralised with 1 M Na_2HPO_4 immediately after the desorption. Figure 1 shows a chromatogram of this purification (UV 280 nm).

50 µl of the antibody fraction purified in this way were analysed on a size exclusion column (SEC, Zorbax 250 GF). 220 mM phosphate buffer, pH 7 + 10% acetonitrile were used as a running agent. As can be seen from the chromatogram of this analysis (Figure 2), the antibody fraction contains IgM and IgG at a ratio of about 3:2. The overall amount of the antibody fraction was about 40 µg (determined by means of size exclusion chromatography (SEC) in comparison with a standard). The antibody fraction obtained in this way was tested in an ELISA with regard to the binding to the antibody 17-1A (which was used as a ligand for the affinity purification): 100 µl aliquots of the antibody which is directed against a tumour-associated antigen (antibody 17-1A; solution with 10 µg/ml in binding buffer) and which was used for the affinity purification were incubated in the wells of a microtiter plate for 1 hour at 37°C. After washing the plate six times with washing buffer A, 200 µl of the blocking buffer

25 FEB 2004

A were added and incubated for 30 minutes at 37°C. After washing the plate as described above, 100 µl aliquots of the affinity-purified antibody fraction to be tested as well as normal human immunoglobulin at the same concentration as a negative control were incubated in dilutions of 1:4 to 1:65,000 in dilution buffer A for 1 hour at 37°C. After washing the plate as described above, 100 µl of the peroxidase-conjugated goat-anti-human-Ig antibody (Zymed) were added at a dilution of 1:1000 in dilution buffer A and incubated for 30 minutes at 37°C. The plate was washed four times with washing buffer A and twice with staining buffer. The binding of the antibodies was detected by adding 100 µl of the specific substrate and the colour reaction was stopped after about 3 minutes by adding 50 µl stop solution. The evaluation was carried out by measuring the optical density (OD) at a wave length of 490 nm (wave length of the reference measurement is 620 nm).

As can be seen in Figure 3, the affinity-purified antibody fraction has significantly bound to the antibody 17-1A whereas normal human immunoglobulin does virtually not bind.

Example 3
Formulation of a vaccine with purified antibodies

The antibody fraction obtained by the affinity purification was formulated with aluminium hydroxide as an adjuvant in accordance with the following instructions:
1 mg aluminium hydroxide (aqueous suspension; Alhydrogel, Superfos) was added to 3 ml of the antibody solution obtained after the affinity chromatography (contains about 40 µg antibodies) and the suspension was centrifuged in a "FILTRON" centrifugation vial (Microsep™, cut-off 10 KD) for 30 minutes at 4000 x g. Subsequently, the solution was suspended twice with 1 ml of the formulation buffer and centrifuged for 30 minutes at 4000 x g. The suspension was filled with formulation buffer to give 0.5 ml and the suspension obtained in this way was filled up in a sterile manner.

Example 4
Immunisation of rhesus monkeys with autologous immunoaffinity-purified antibodies

The respective rhesus monkey from the serum of which the vaccine was obtained as explained in Examples 2 and 3 was vaccinated subcutaneously in the back with this vaccine. Before this first vaccination, 5 ml blood were taken for recovery of serum (for determining the starting value for the characterisation of the immune response). Two

25 FEB 2004

weeks after that, 10 ml blood were taken again for recovery of serum. With 4 ml of this serum, the recovery of an autologous vaccine was repeated as has been described above. The rhesus monkey was injected the newly obtained vaccine again subcutaneously in the back. 4 weeks after this vaccination, again 5 ml blood were taken for recovery of serum (for determining the effect of the two vaccinations).

The pre-serum of this rhesus monkey and the immune serum 4 weeks after the second vaccination were analysed in a cell ELISA for whether antibodies bind on the KATO III cell line. For this purpose, the following steps were carried out:

The wells of a microtiter plate were incubated with 100 µl of a cell suspension of the KATO III cell line at a concentration of $2x10^6$ cells/ml in medium A over night at +37°C. After sucking off the supernatant, the plate was incubated with 50 µl fixing solution per well for 5 minutes at room temperature. After sucking off the supernatant, 200 µl blocking buffer B were added to each well and the plate was incubated for 1 hour at room temperature. After washing it twice with 200 µl washing buffer B, 100 µl aliquots of the monkey serums to be tested were incubated in dilutions of 1:4 to 1:56,000 in dilution buffer B for 1 hour at 37°C. After washing the plate twice with 100 µl ice-cold washing buffer B, 100 µl of a peroxidase-conjugated goat-anti-human-Ig antibody (Zymed) in a dilution of 1:1000 in dilution buffer A were added and incubated for 45 minutes at 37°C. The plate was washed three times with 100 µl ice-cold washing buffer B. The binding of the antibodies was detected by adding 100 µl of the specific substrate and the colour reaction was stopped after about 5 minutes by adding 50 µl stop solution. The evaluation was carried out by measuring the optical density (OD) at a wave length of 490 nm (wave length of the reference measurement is 620 nm).

As can be seen from Figure 4, there are immunoglobulins in the immune serum of the rhesus monkey which was vaccinated in an autologous manner, these immunoglobulins being able to bind on Kato III tumour cells, whereas such antibodies can hardly be detected in the pre-serum of the same animal.

Due to the results of the above-described experiments, it could be shown exemplarily that the vaccination with individual autologous antibody fractions which were obtained by immunoaffinity purification on a monoclonal antibody directed against a tumour-associated antigen triggered a humoral immune response which binds on human tumour cells expressing said tumour-associated antigen.

Example 5:

Preparation of an immunoaffinity column with two different TAA-specific antibodies

25 FEB 2004

A monoclonal mouse antibody which reacts with EP-CAM and a monoclonal mouse antibody which reacts with Lewis Y were both coupled to activated CH sepharose 4B (Pharmacia Biotech, Sweden) according to standard techniques:
7.5 g CH sepharose 4B were suspended in 20 ml 1 mM HCl for 15 minutes and soaked. The gel was washed with 1 l 1 mM HCl and then with 200 ml coupling buffer on an AG3 filter of sintered glass. 100 mg murine antibody (stock solution 10 mg/ml) were dialysed against 5 l coupling buffer and adjusted to 5 mg/ml with coupling buffer. This solution was mixed with the gel suspension in a closed container. A suspension which is suitable for coupling can be achieved with a ratio of gel to buffer of 1:2. This suspension was rotated for 24 hours at 4°C. Then, the excess of the ligand was washed off with 3 x 30 ml coupling buffer. The remaining reactive groups were blocked by an 1-hour incubation with 1 M ethanol amine at 4°C. Then, the gel was rotated for 1 hour at room temperature with 0.1 M Tris-HCl buffer. Finally, the gel was washed with three cycles of buffers with alternating pH values. Every cycle consists of 0.1 M sodium acetate buffer, pH 4 with 0.5 M NaCl, and subsequently 0.1 M Tris-HCl buffer, pH 8 with 0.5 M NaCl. The gel was stored at 4°C.
Two identical volumes of the two affinity matrices were mixed and packed as an immunoaffinity chromatography column (System Äkta, Pharmacia, Sweden).

Example 6:
Purification of antibodies from monkey serum by simultaneous immunoaffinity purification with two different TAA-specific antibodies

About 20 ml blood each were taken from immunologically naïve rhesus monkeys. 9 ml serum each were purified using the immunoaffinity chromatography column described in Example 5 (System Äkta, Pharmacia, Sweden).

Buffer for application: purification buffer A
Elution buffer: purification buffer B

The eluate was neutralised with 0,5 M sodium hydrogen phosphate solution.

The eluates were fractionated in such a way that the purified proteins are present at a high concentration.
50 µl of the purified antibody fraction were analysed on a size exclusion column (SEC, Zorbax 250 GF). 220 mM phosphate buffer, pH 7 + 10% acetonitrile were used as a running agent (flow: 1.000 ml/min; wave length 214 nm). As can be seen from the chromatogram of this analysis (Figure 5), the antibody fraction contains IgM (retention time 6.963 min) and IgG (retention time 8.745 min) at a ratio of about 3:2.

25 FEB 2004

The overall amount of the antibody fraction was about 50 µg (determined by means of SEC in comparison with a standard, Figure 6).

Example 7:
Different formulations of a vaccine from purified antibodies

The vaccine was formulated in ultrafiltration units for centrifugation (Centricon 10; Amicon, USA). For this purpose, the ultrafiltration units were washed by centrifugation with 1 mM sodium phosphate buffer, 0.86% NaCl, pH 6.0 (NBK).
Then, 1 ml buffer (NBK) was filled into the unit and 37 µl Alhydro gel 2% (Superfos Biosector, Denmark) were added.
After adding the neutralised eluate of Example 6, centrifugation and washing (with 5 ml buffer) were carried out according to the Centricon instructions.
Then, the vaccine was resuspended, filled to 550 µl with buffer and filled up in a sterile manner.
Alternatively, the vaccine was filled to 545 µl with buffer after resuspension and 5.5 µl thimerosal stock solution (10 mg /ml, Sigma, USA) were added.
A third variant of the formulation of the vaccine was heat-denatured in an autoclave (121°C; 20 min) after being filled in glass vials in a sterile manner.

Claims

1. Use of antibodies for the production of a pharmaceutical composition which is suitable as a vaccine for the therapeutic of prophylactic vaccination against cancer characterised in that the antibodies are recovered from body fluids containing antibodies by immunoaffinity purification, wherein antibodies recognising one or more tumour-associated antigens or fragments thereof having the same idiotype are used as ligands for the immunoaffinity purification and wherein the body fluids are derived from an individual that has not been administered a monoclonal antibody before which is directed against one of the tumour-associated antigens.

2. The use according to claim 1, wherein the pharmaceutical composition is intended for administration to the individual from the body fluid of which the antibodies were obtained in the sense of an autologous, individual vaccine.

3. The use according to any one of claims 1 to 2, wherein the body fluid is serum.

4. The use according to any one of claims 1 to 3, wherein the individual is a human.

5. The use according to any one of claims 1 to 4, wherein several antibodies directed against different tumour-associated antigens and/or against different epitopes of such antigens are used at the same time for the immunoaffinity purification.

6. The use according to any one of claims 1 to 5, wherein the antibodies used as ligands for the immunoaffinity purification are monoclonal antibodies.

7. The use according to any one of claims 1 to 6, wherein the pharmaceutical composition is prepared repeatedly and is intended for subsequent administration as an individual vaccine repeated in suitable intervals.

8. The use according to any one of claim 1 to 7, wherein the tumour-associated antigens are membrane-located molecules which are frequently expressed and/or co-expressed on cancer cells of epithelial origin or on cancer cells of neuroendocrine origin or on cancer cells of the blood-forming system.

9. The use according to any one of claim 1 to 8, wherein the antibodies purified by immunoaffinity purification or the derivatives thereof are formulated together with a suitable vaccine adjuvant.

10. The use according to any one of claims 1 to 9, wherein the composition furthermore contains a protein which enhances the immune response and which is administered at the same time as the antibodies.

11. The use according to claim 10, wherein the protein is a human protein.

12. The use according to claim 11, wherein the human protein is granulocyte-macrophage stimulating factor (GM-CSF).

13. The use according to any one of claims 1 to 12, wherein the composition is suitable for administration of the antibodies purified by immunoaffinity purification as a vaccine by way of subcutaneous or intramuscular injections.

14. The use according to any one of claims 1 to 13, wherein the antibodies contained in the composition are mixed with an adjuvant and subjected to a heat treatment.

15. Use of isolated, ex vivo cultivated dendritic cells of an individual for the production of a pharmaceutical composition which is suitable as a vaccine for the therapeutic or prophylactic vaccination against cancer, wherein the dendritic cells have been incubated before in vitro with antibodies which have been obtained from an antibody-containing body fluid of the same individual by means of immunoaffinity purification, wherein antibodies recognising one or more tumour-associated antigens or fragments thereof having the same idiotype are used as ligands for the

25 FEB 2004

immunoaffinity purification and wherein the body fluid is derived from an individual that has not been administered a monoclonal antibody before which is directed against one of the tumour-associated antigens.

16. A pharmaceutical composition containing IgM and IgG antibodies obtained from body fluids containing antibodies by means of immunoaffinity purification, wherein antibodies recognising one or more tumour-associated antigens or fragments thereof having the same idiotype are used as ligands for the immunoaffinity purification and wherein the body fluids are derived from an individual that has not been administered a monoclonal antibody before which is directed against one of the tumour-associated antigens.

17. A pharmaceutical composition containing isolated, ex vivo cultivated dendritic cells of an individual, wherein the dendritic cells have been incubated before in vitro with antibodies which have been obtained from an antibody-containing body fluid of the same individual by means of immunoaffinity purification, wherein antibodies recognising one or more tumour-associated antigens or fragments thereof having the same idiotype are used as ligands for the immunoaffinity purification and wherein the body fluid is derived from an individual that has not been administered a monoclonal antibody before which is directed against one of the tumour-associated antigens.

18. A method for the production of an antibody preparation characterised in that antibodies are recovered from a body fluid containing antibodies by immunoaffinity purification, wherein antibodies recognising one or more tumour-associated antigens or fragments thereof having the same idiotype are used as ligands for the immunoaffinity purification and wherein the body fluid is derived from an individual that has not been administered a monoclonal antibody before which is directed against one of the tumour-associated antigens.

19. The method according to claim 18, wherein several antibodies directed against different tumour-associated antigens and/or epitopes of such antigens are used at the same time for the affinity purification.

20. The method according to claim 18 or 19, wherein the antibodies used for the affinity purification are monoclonal antibodies.

21. The method according to any one of claims 18 to 20, wherein the body fluid is serum.

22. A method for the production of a pharmaceutical composition characterised in that an antibody preparation is prepared in accordance with a method according to any one of claims 18 to 21 and the antibody preparation obtained in this way is subsequently formulated with a pharmaceutically acceptable carrier and/or a suitable vaccine adjuvant.

23. The method according to claim 22, wherein the antibody preparation is mixed with an adjuvant and, subsequently, subjected to a heat treatment.

24. The method according to any one of claims 18 to 21, wherein the immunoaffinity purification is the only purification step.