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(54) HUMAN LYMPHOID TISSUE IN AN IMMUNOCOMPROMISED HOST
MENSCHLICHES LYMPH-GEWEBE IN EINEM WIRT MIT ANGEGRIFFENEM IMMUNSYSTEM
TISSU LYMPHOIDE HUMAIN UTILISE CHEZ UN HOTE PRESENTANT UN DEFICIT IMMUNITAIRE

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• Science, Volume 241, Issued 23 September 1988, MCCUNE et al., "The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function", pages 1632-1639, see the entire document, and especially the left column on page 1639.
• Current Topics in Microbiology and Immunology, Volume 152, Issued 1989, MCCUNE et al., "The SCID-hu mouse: current status and potential applications", pages 183-193, see the entire document, and especially pages 188, 191 and 192.
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INTRODUCTION

Technical Field

The field of this invention is the utilization of the human lymphoid system in testing human immune response and for the production of human antibodies.

Background

The human immune system is the guardian of human health against invasive organisms, aberrant cellular conditions, e.g. neoplasia, and the like. There is either or both a humoral and/or cellular response to a variety of pathological conditions. In the prophylactic or therapeutic treatment of these conditions, the primary focus is on the effect of the treatment on the disease state. In many cases, the treatment may have an advantageous or detrimental effect on the immune response to the condition. Studying such response is difficult until the therapy is used with human hosts.

For a number of diseases, vaccinations are provided. Frequently, there is no animal model which can provide the desired information concerning the nature of the response to the vaccine, the efficiency of the response and the overall effect of the vaccine on the host. In many instances the inability to evaluate the vaccine with animals may preclude its use as a vaccine.

In light of the uncertainties concerning therapies for humans, there is a substantial need to develop animal models which will allow for the testing of the effect of therapies on the human immune system.

The monoclonal antibody, discovered in the middle 1970’s has been exploited widely in diagnostics and substantially less so in therapy. It is based on the observation that a mammalian host can be induced to make a primary immune response against any antigen by selective immunization and that the specific B cells making antibody can be immortalized by fusion to create hybridoma cell lines producing monospecific antibody against the immunogen. The original promise of a “magic bullet” has not been realized. One of the limitations on the use of monoclonal antibodies for therapy has been the immune response to heterologous antibodies. Since, for the most part, mouse antibodies are the most convenient to induce and mouse B-lymphocytes to immortalize for production of monoclonal antibodies, with few exceptions, the monoclonal antibodies which have found commercial and research use have been mouse monoclonal antibodies. The ability to prepare human monoclonal antibodies has been limited due to the inability to induce a primary immune response in vitro. There is, therefore, interest in being able to develop other techniques for producing human monoclonal antibodies, where the opportunity exists to produce antibodies of high affinity, specific for epitopes of interest, and having the human constant region and framework region of the polymorphic region.

Relevant Literature

EPO 0 322 240 describes the introduction of human fetal liver, thymus and lymph node into an immunocompromised mouse for use in initiating cellular and/or tissue response under conditions where the response may be evaluated to determine the efficacy of the agents and/or conditions. EPO 0 379 554 describes the introduction of human adult peripheral blood leukocytes into an immunocompromised mouse wherein immunologic function is reconstituted. The production and viral infection of chimeric immunocompromised mice reconstituted with human fetal lymphoid tissue is further discussed in McCune et al. (1988) Science 241:1632; Yancopoulos and Alt (1988) Science 241:1581 and McCune et al. (1989) Current Topics in Microbiology and Immunology 152:183.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

A chimeric host comprising human fetal lymphoid solid tissue in an immunocompromised non-primate host is provided, where the lymphoid tissue is characterized by comprising at least antigen presenting cells, particularly B-lymphocytes, and T-lymphocytes, including CD4 and normally CD8 cells. The solid tissue may be used in various methodologies utilizing the immune system for the production of secreted products or for evaluating the response of the immune system, particularly the lymphoid system, although the myelomonocytic system may also be involved, to a compound or method of treatment for a diseased state which is responsive to the immune system. A single organ is used rather than a combination of interacting organs, where cells may traffic from one organ to another.

The chimeric host has a number of uses based on the presence of viable functioning human fetal lymphoid tissue in the chimeric host. The presence of antigen presenting cells and T-lymphocytes provides for the opportunity to immunize with an antigen and for the production of B-lymphocytes having the immunoglobulin locus rearranged to produce immunoglobulins having specificity for a predetermined antigen. The presence of the cells of the lymphoid lineage, particularly in conjunction with cells of the myelomonocytic lineage, allows for the evaluation of compounds and methodologies on the modulation of the immune response, up or down regulating the production of cells involved with the immune response. Thus drugs, combinations of drugs, and treatment modalities may be evaluated as to their effect on the expansion and/or inhibition of the proliferation of cells involved with the immune response.

The first aspect of the subject invention to be considered will be the production of human monoclonal antibodies. Methodology is provided for creation of human
activated B-lymphocytes in vivo to any antigen and the use of the B-lymphocytes for production of human monoclonal antibodies to a predetermined epitope. Lymphoid tissue is introduced at an appropriate site of an immunocompromised mammal. Sufficient time is allowed for the lymphoid tissue to become vascularized and lymphatic vessels connected. B-lymphocytes are then activated with an appropriate immunogen having the desired epitope to stimulate B-lymphocytes producing immunoglobulin specific for the desired epitope. After a sufficient degree of stimulation, the B-lymphocytes may then be cloned or immortalized for continuous production of monoclonal antibodies. The resulting monoclonal antibodies may be screened for their binding affinity and the immortalized or cloned cells secreting the antibodies maintained by any convenient means.

Various lymphoid or other lymphocyte containing human tissue may be employed which provides for B-lymphocyte and T-lymphocyte interaction, preferably including macrophage interaction. Tissue which may be employed includes lymph node, both mesenteric and peripheral, either with or without mesentery, thymus, tonsil, spleen, pancreas, bone, etc., preferably lymph node. The tissue is preferably obtained from a fetal source, having a gestational age of at least four weeks, more usually at least about 6 weeks and ranging up to neonate tissue, depending upon the nature of the tissue or organ, preferably being fetal tissue of about 7 to 22 weeks.

For different organs, differently aged tissue may be preferred. For example, for human lymph node, the age is desirably equal or greater than about 15 gestational weeks (g.w.), preferably 16-20 g.w.; for human thymus, from about 9 to 22 g.w., preferably less than about 20 g.w. Fetal liver tissue may find use in combination with other tissue, particularly lymph node tissue, where the lymph node tissue will generally be of an age in the range of 9-22 g.w. For bone marrow, the tissue will be about 12 to 24 g.w.

The xenogenic host will be an immunocompromised mammal other than human. The immunocompromised host may be immunocompromised in a variety of ways, where the result will be the substantially lack of functional T- and B-lymphocytes. The xenogenic host may have defects at various levels resulting in an immunocompromised host. The defect results in loss of functional antibody secreting lymphocytes, particularly the inability to produce antibody, as a result of a deficit in the rearrangement of the immunoglobulin or T-lymphocyte locus, in factor receptors, in T-lymphocytes, or the like.

Any mammalian host may be employed other than the presently available SCID mice and horses (equine), which host may include members of the ovine, bovine, caprine, lagomorpha, primate (other than human), porcine, canine, feline, etc. Animal hosts of particular interest are laboratory animals, such as mice, rats, guinea pigs, e.g. capybara, rabbits, as well as domestic animals, such as primates other than humans, cows, sheep, pigs, horses, or the like. Of more particular interest are those mammals which lack functional B- and T-lymphocytes as a result of a genetic defect in the ability of the lymphoid lineage to differentiate and mature from a progenitor cell. That is, the animals lack functioning lymphoid cells because of other than a lack of a thymus providing for processing of lymphoid cells. These mammals are illustrated by the CB-17 scid/scid mice. If desired, the immunocompromised host may be produced by changes induced by transformation of embryonic stem cells or introduction of transformed hematopoietic stem cells in a host lacking functional bone marrow, e.g. a lethally irradiated host. The defect may be any of a large number of genes, such as a recombinase gene, a regulatory gene for the recombinase, a gene involved in transport of the immunoglobulin, loss of exons associated with the constant regions, a lesion in the loci site for the T cell receptor and the surface immunoglobulin or the like.

Various sites may be selected for the introduction of the human tissue, where the sites are downstream from a convenient site in the blood or lymphatic system for introduction of the immunogen. Sites which have found application include the popliteal fossa, the mammary fat pad, particularly the fourth mammary fat pad, cervical and the like. Of particular interest is the popliteal fossa, where the immunogen may be introduced in the footpad and drained by the lymphatic vessel to the popliteal fossa, particularly on implanted lymph node.

The tissue may be fresh tissue, obtained within about 48 hours of death, or freshly frozen tissue, tissue frozen within about 12 hours of death and maintained at below about -10 degrees C, usually at about liquid nitrogen temperature (-70 degrees C) indefinitely. The tissue may be from an organ implanted in a chimeric host, where the tissue may be removed from 2 to 4 weeks after implantation, or longer. In this manner, the tissue originally obtained from the host source may be greatly expanded, substantially increasing the total number of chimeric hosts which may be obtained. The tissue obtained from the chimeric host may be treated analogously to the tissue obtained from the human source. The tissue may be provided as portions of organs or complete organs, comprising or freed of attached stromal elements, generally from about 0.5 to 4 mm, more usually from about 1 to 2 mm, so that the sections can easily fit into a trocar used for implantation, usually conveniently of about 15-to 20-gauge. Normally, the tissue will not have been subject to culture in vitro for an extended period of time. In some cases, whole organ grafts may be transplanted by anastomosing donor and host blood vessels, lymphatic vessels, and the like.

Besides the lack of functional T- and B-lymphocytes, further reduction in immunocompetence may be achieved by breeding, use of natural killer deficient mutants, irradiation of the host or use of immunotoxoid labels joined to antibodies specific for these cells.
to be killed, for example, lymphoid or myelomonocytic lineages. Particularly, where immunocompetence is to varying degrees provided by the tissue being introduced into the host in accordance with the subject invention, native immunocompetence can be further reduced below the low level naturally present in the host.

The host will usually be of an age less than about 25 percent of the normal lifetime of an immunocompetent host, usually about 1 to 20 percent of the normal lifetime. Generally the host will be at least about 3-week old and large enough to manipulate for introduction of the tissue at the desired site. For example, mice which may be considered to have about 2 to 4 year lifetime are used at about 3 to 10, usually 4 to 8 weeks of age. Growth of the tissue within the host will vary with the organ, usually being at least about 1 to 2 fold.

Normally, the tissue which is introduced into the host will be allowed to grow and vascularize and have lymphatic vessels connected before immunization. Generally, at least one week will transpire, preferably at least about 2 weeks, and usually immunization will occur within 20 weeks of transplantation, more usually within 2 to 10 weeks of transplantation.

The subject methodology may be used with any compound having an epitope of interest, including epitopes common to humans. Since there is no concern about the effect of producing antibodies specific to a human protein in the subject chimeric host, one can develop antibodies to native human proteins. Immunogens of interest may be both haptens and antigens, where the haptens are modified to provide for an immune response. Compounds of interest may include small synthetic organic molecules, generally of less than about 5 kD (kilodaltons), usually less than about 2 kD, polypeptides and proteins, lipids, saccharides, and combinations thereof. The compounds may be synthetic or naturally occurring, including drugs, hormones, cytokines, surface membrane proteins, enzymes, sugar side groups, toxins, etc. The immunogen may be combined with a wide variety of adjuvants, such as complete Freund's adjuvant, specol B, pertussis or its toxin, etc. Usually, the injection will vary widely depending upon the size of the animal, usually varying from about 10 ml to 5 ml, comprising from about 10 mg to 1 mg of the immunogen. The adjuvants will be used in conventional amounts in accordance with the nature of the adjuvant.

Administration will normally be by injection, which will usually be subcutaneous, intramuscular, intraperitoneal or intravascular, where the injection is upstream from the site of the human lymphoid tissue. One or more booster injections may be made, usually within 1 to 6, more usually 2 to 4 weeks of the previous injection, where a booster injection may have the same composition or different composition from the prior injection, by changing the concentration, adjuvant, or the like. In conjunction with administration of the immunogen, IL-6 may be administered, generally to provide a concentration in the bloodstream in the range of about 0.5-20 mg/ml.

After the immunization is complete, the tissue may be harvested and the B-lymphocytes immortalized and/or cloned as appropriate. Various fusion partners are available, which are capable of immortalizing human B-lymphocytes. See, for example, Ken-Mitchel et al., Clin. Lab. Anal., 1989, 3:41-9. The methods employed for the fusion are to combine the B-lymphocytes with the fusion partner in the presence of a fusogen, usually a nonionic detergent for sufficient time for fusion to occur, followed by selection of the resulting hybridomas in accordance with the nature of the marker(s) present in the fusion partner. The cells may then be subjected to limiting dilution to provide for clones free of contaminated cells, so as to result in a homogeneous antibody composition. The hybridomas may then be introduced into host animals, e.g., mice or rats, to produce ascites fluid or mechanically expanded, using spinner flasks, roller bottles, etc. The host will be immunocompromised, so as to be able to accept the neoplastic graft.

The resulting antibodies may be used in a variety of ways, both diagnostic and therapeutic. However, since other antibodies which are normally more easily obtained, such as non-human antibodies can be used in in vitro diagnostics, for the most part the subject antibodies will be used for in vivo diagnostic and therapeutic use in humans. Thus, the subject antibodies may be used in the treatment of septicemia, ablation of particular T-lymphocyte receptors, neutralizing viruses or other pathogens, for in vivo diagnoses, for targeted toxicity against neoplastic cells or precursors to such cells, passive immunization, in conjunction with transplantation, and the like. The subject antibodies may be modified by radio-labeling, conjugation to other compounds, such as biotin, avidin, enzymes, cytotoxic agents, e.g., ricin, diphtheria toxin, arbin, etc., and the like.

The subject chimeric hosts may also be used in the production of human T-lymphocytes specific for a particular target cell or a particular immunodominant sequence. These T-lymphocytes may be CD4 helper cells, CD8 suppressor cells, natural killer cells, cytotoxic T-lymphocytes, antibody dependent cytotoxic cells, tumor infiltrating lymphocytes ("TILs"), etc. The same system that is employed for the production of B-lymphocytes specific for a predetermined antigen may be employed for the production of CD4 helper cells specific for an immunodominant sequence which binds the target major histocompatibility complex to which the T-lymphocyte is restricted. Also, for other types of T-lymphocytes, by providing for the appropriate stimulus, one may produce TILs, using neoplastic tissue in conjunction with the lymphoid tissue, for other types of T-lymphocytes, using various cytokines and/or growth factors in conjunction with a stimulus, e.g., microorganism, and the like.

The lymphoid tissue may also be used in the studies of vaccines and drugs, as to efficacy in producing an immune response and as to the effect of the drug on the immune system. For a vaccine, lymphoid tissue, such as lymph node, may be used in substantially the same
manner as was described for the production of monoclonal antibodies. By providing a base line for response with a number of different vaccines one can compare vaccines as to their response in producing a primary and secondary immune response. In addition, one can immortalize the activated B-lymphocytes and screen the antibodies for their protective effect against the pathogen.

In addition, one can use the lymphoid tissue to determine the effect of drugs on the immune system. One can detect the effect of drugs on the viability of human hematopoietic cells in various tissues, the effect of the drugs upon stimulation by immunogens and/or cytokines and the like. By introducing bone comprising bone marrow in the immunocompromised host, determining the relative population of the cells in the bone marrow and the change in the population in the presence and absence of a therapeutic dosage of the drug, one can obtain an indication of the effect of the drug on the hematopoietic cells in the bone marrow. By stimulating the bone marrow with an immunogen in the presence and absence of the drug and evaluating the immune response, one can measure the effect of the drug on the humoral immune response. To measure the effect, one could determine the number of cells which have been activated to produce slg. by preparing tissue slices, labeling the slg for the immunogen and counting the number of cells which are specifically labeled.

The following examples are offered by way of illustration and not by way of limitation.

**EXPERIMENTAL**

CB-17 scid/scid mice were transplanted with mesenteric lymph nodes into the popliteal fossa region. An incision is made in the site directly over the fat pad on the back hind leg of the mouse. The main vein that runs vertically up to the trunk that rests on top of the fat pad is cauterized. An incision is made through the fat to expose the popliteal fossa lymph node (LN). The LN is removed and the human tissue is inserted at the same site. Sutures are made to close the dermal incision.

A dose of 50 mg of trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH) combined with specol adjuvant (5 ml TNP-KLH [10 mg/ml] is vortexed with 6 ml specol adjuvant) was injected subcutaneously into the footpad, where the lymphatic vessels drain into the popliteal fossa lymph node. The graft in one mouse showed appropriate cellularity and was observed to contain a large number of human IgG and IgM positive cells. After 4 days from the immunization, the tissue was harvested, histologic sections prepared and these sections analyzed. The staining procedure is as follows. The immunized human graft is surgically removed from the host and snap frozen in liquid nitrogen. Tissue is sectioned 8 mm thick using a cryostat and placed on glass slides. Tissue is stored if not used promptly at -20 degrees C. The slides are then wet 1 x PBS and incubated with trinitrophenyl-conjugated alkaline phosphatase (TNP-AP) diluted in 0.1% BSA/PBS and containing 1% normal human serum. The slides are washed 3x in 1 x PBS. The development is performed using the substrate solution: naphthol-As-phosphate, fast Blue BB salt dissolved in DMF diluted in 0.05 propional buffer, pH 9.75, and 10 mM levamisole-HCl. The reaction is allowed to proceed for 5-10 min and stopped by submerging the slide in 1 x PBS. The slides are then counterstained with hematoxylin for 30 sec, dried and mounted with a coverslip using glycerol/gelatin.

When stained for anti-TNP producing cells, there were several very distinct positive cells. Positive cells show a blue cytoplasmic staining. The staining was shown not to be due to indigenous alkaline phosphatase by the development of the tissue with substrate alone. Blocking of the staining was also shown using 10 mg/ml of TNP-KLH, while 1 mg/ml did not inhibit the cellular staining. The double staining showed human IgM positive cells specific for TNP.

**Human fetal bone implantation**

Human fetal long bones (17-22 g w.) of about 1 cm in length (1-2 bones) were transplanted subcutaneously into CB 17 scid/scid mice. At different time points after transplantation, bones were taken out and cells recovered from them were stained with either human specific antibody, MEM-43, or mouse specific antibody, Ly5.1 and analyzed by FACS or cytoxin preparations. Sections from the transplants were prepared for routine histology.

Human hematopoiesis was not observed by histology or by cytoxin preparations at 2-3 weeks after transplantation. The majority of cells recovered from human bone grafts were positive for MEM-43. Scatter analysis by flow cytometry did not show lymphoid or myeloid populations, suggesting that the majority of cells were non-hematopoietic in origin.

At 4-5 weeks after transplantation, signs of hematopoiesis (i.e., presence of blast cells, immature forms of myelomonocytic cells and erythroid blasts) were observed in cytoxin preparations in most cases analyzed. The MEM-43 positive cells in these samples showed a scatter profile similar to that of the fetal bone marrow samples. Cells of the myelomonocytic lineage, B cell lineage and the erythroid lineage were shown by immunofluorescent staining with LeuM1 (CD15), CD10 and CD19, and anti-human glycophorin A, respectively.

The above data demonstrated that human hematopoiesis is maintained inside the transplanted bones for periods of time of at least 8 weeks after implantation in the absence of other human tissue, that cells can be detected of the various lineages, and in view of the availability of human cells in the bone tissue, the effect of various compounds or conditions may be investigated in relation to the maintenance and proliferation of the
hematopoietic cells.

It is obvious from the above results that the subject methodology provides for a unique way to achieve human monoclonal antibodies against any epitope of interest. Animals may be selected which are easy to handle, can be readily immunized, and the tissue harvested in accordance with conventional techniques. By employing booster shots and using a plurality of animals, a large variety of antibodies may be obtained of high affinity specific for a particular epitope or epitopes of a particular antigen. The resulting stimulated B-lymphocytes may then be used for immortalization and/or cloning to provide for a stable supply of human monoclonal antibodies.

Claims

1. A method for producing human antibodies, said method comprising:

   immunizing an immunocompromised chimeric mouse host comprising human fetal lymphoid tissue with an immunogen at a site upstream from vascularized and lymphatic vessel-connected human fetal lymphoid tissue comprising B- and T-lymphocytes; and

   harvesting said human lymphoid tissue as a source of B-lymphocytes producing antibodies to said immunogen;

   wherein said chimeric mouse host is characterized by being immunocompromised in at least substantially lacking B- or T-lymphocytes, comprising vascularized and lymphatic vessel connected human fetal lymphoid tissue comprising human B- and T-lymphocytes as a result of implantation of said human fetal lymphoid tissue at a site other than the kidney capsule, wherein said site is a vascularizable and lymphatic vessel connectable site, and capable of at least a primary immune response to an immunogen.

   6. A method according to Claim 5, wherein said B-lymphocytes are immortalized by fusion or Epstein-Barr transformation prior to cloning.

   7. A method according to Claim 5, wherein said vascularizable and lymphatic vessel connectable site is the popliteal fossa.

   8. A method according to Claim 5, wherein said immunizing is injection in the footpad drained by said vascularizable and lymphatic vessel connectable site.

   9. A method according to Claim 5, wherein said fetal lymph node tissue is whole fetal lymph node.

Patentansprüche

1. Verfahren zur Herstellung menschlicher Antikörper, mit den Schritten:

   Immunisierung eines chimären Mauswirts mit angegriffenem Immunsystem, der menschliches fetales Lymph-Gewebe enthält, mit einem Immunogen an einem Ort oberhalb (bezogen auf die Flußrichtung) von vaskularisiertem und Lymphgefäßerfordenem menschlichen feta- len Lymph-Gewebe, das B- und T-Lymphozyten umfaßt;

   Gewinnung des menschlichen Lymph-Gewe- bes als Quelle für B-Lymphozyten, die Antikörper zu diesem Immunogen herstellen;

   wobei der chimäre Mauswirt dadurch gekenn- zeichnet ist, daß er ein angegriffenes Immun-
system mit zumindest einem beträchtlichen Mangel an B- oder T-Lymphozyten hat, daß er vaskularisierter und Lymphgefäß-verbindungsmäßiges menschliches fötales Lymph-Gewebe enthält, das menschliche B- und T-Lymphozyten als eine Folge der Implantation dieses menschlichen fötalen Lymph-Gewebes an einem anderen Ort als der Nierenkapsel enthält, wobei dieser Ort ein vaskularisierbarer und Lymphgefäß-verbindbarer Ort ist und daß er wenigstens zu einer primären Immunantwort auf ein Immunogen in der Lage ist.

2. Verfahren nach Anspruch 1, bei dem das Lymphgewebe ein Lymphknoten ist.

3. Verfahren nach Anspruch 1, bei dem der vaskularisierte und durch Lymphgefäße verbindbare Ort die Kniekehle (popliteal fossa) ist.

4. Verfahren nach Anspruch 1, bei dem die Maus eine C.B-17 scid/scid Maus ist.

5. Verfahren zur Herstellung menschlicher, monoklonaler Antikörper mit den Schritten:

   Immunisierung eines chimären Mauswirts mit angegriffenem Immunsystem, der menschlichen, fötalen Lymph-Gewebe enthält, mit einem Immunogen an einem Ort oberhalb (bezogen auf die Flüssrichtung) von vaskularisiertem und Lymphgefäßverbundenem menschlichen, fötalen Lymph-Gewebe, das B- und T-Lymphozyten umfaßt;

   Gewinnung des menschlichen, fötalen Lymph-Gewebes als Quelle für B-Lymphozyten, die Antikörper zu diesem Immunogen herstellen;

   Klönnen von Immunoglobulin produzierenden B-Lymphozyten zur Herstellung menschlicher, monoklonaler Antikörper, die für ein Epitop dieses Immunogens spezifisch sind;

   wobei der chimäre Mauswirt dadurch gekennzeichnet ist, daß er ein angegriffenes Immunsystem mit zumindest einem beträchtlichen Mangel an B- oder T-Lymphozyten hat, daß er vaskularisierter und Lymphgefäß-verbindungsmäßiges menschliches, fötales Lymph-Gewebe enthält, das menschliche B- und T-Lymphozyten als eine Folge der Implantation dieses menschlichen, fötalen Lymph-Gewebes an einem anderen Ort als der Nierenkapsel enthält, wobei dieser Ort ein vaskularisierbarer und Lymphgefäß-verbindbarer Ort ist und daß er wenigstens zu einer primären Immunantwort auf ein Immunogen in der Lage ist.


7. Verfahren nach Anspruch 5, bei dem der vaskularisierbare und Lymphgefäß-verbindbare Ort die Kniekehle (popliteal fossa) ist.


Revendications

1. Procédé de production d’anticorps humains, ledit procédé comprenant:

   l’immunisation d’une souris hôte chimérique présentant un déficit immunitaire, comprenant du tissu lymphoïde foetal humain avec un immunogène à un site en amont de tissu lymphoïde foetal humain vascularisé connecté aux vaisseaux lymphatiques comprenant des lymphocytes B et T, et la récolte dudit tissu lymphoïde humain comme source de lymphocytes B produisant des anticorps contre ledit immunogène;

   dans lequel ladite souris hôte chimérique est caractérisée par le fait qu’elle présente un déficit immunitaire au moins au niveau des lymphocytes B ou T sensiblement absents, qu’elle comprend du tissu lymphoïde foetal humain vascularisé connecté aux vaisseaux lymphatiques comprenant des lymphocytes humains B et T par suite de l’implantation dudit tissu lymphoïde foetal humain à un site autre que la capsule des reins, dans lequel ledit site est un site vascularisable et connectable aux vaisseaux lymphatiques, et capable d’au moins une réponse immunitaire primaire à un immunogène.

2. Procédé selon la revendication 1, caractérisé en ce que ledit tissu lymphoïde est un ganglion lymphatique.

3. Procédé selon la revendication 1, caractérisé en ce que ledit site vascularisable et connectable aux vaisseaux lymphatiques est la fosse poplitée.

4. Procédé selon la revendication 1, caractérisé en ce que ladite souris est une souris C.B-17 scid/scid.
5. Procédé de production d'anticorps monoclonaux humains, ledit procédé comprenant :

l’immunisation d’une souris hôte chimérique présentant un déficit immunitaire, comprenant du tissu lymphoïde foetal humain avec un immuno-gène à un site en amont de tissu lymphoïde foetal humain vascularisé connecté aux vaisseaux lymphatiques comprenant des lymphocytes B et T, et la récolte dudit tissu lymphoïde foetal humain comme source de lymphocytes B produisant des anticorps contre ledit immunogène ; et

le clonage de lymphocytes B producteurs d’immunoglobuline pour la production d’anticorps monoclonaux humains spécifiques d’un épitope dudit immunogène ;

dans lequel ladite souris hôte chimérique est caractérisée par le fait qu’elle présente un déficit immunitaire au moins au niveau des lymphocytes B ou T sensiblement absents, qu’elle comprend du tissu lymphoïde foetal humain vascularisé connecté aux vaisseaux lymphatiques comprenant des lymphocytes humains B et T par suite de l’implantation dudit tissu lymphoïde foetal humain à un site autre que la capsule des reins, dans lequel ledit site est un site vascularisable et connectable aux vaisseaux lymphatiques, et capable d’au moins une réponse immunitaire primaire à un immunogène.

6. Procédé selon la revendication 5, caractérisé en ce que lesdits lymphocytes B sont immortalisés par fusion ou par transformation par le virus Epstein-Barr préalablement au clonage.

7. Procédé selon la revendication 5, caractérisé en ce que ledit site vascularisable et connectable aux vaisseaux lymphatiques est la fosse poplitée.

8. Procédé selon la revendication 5, caractérisé en ce que ladite immunisation consiste en une injection dans le coussinet de la patte irrigué par ledit site vascularisable et connectable aux vaisseaux lymphatiques.

9. Procédé selon la revendication 5, caractérisé en ce que ledit tissu de ganglion lymphatique foetal est l’ensemble du ganglion lymphatique foetal.