MHC class II is found on macrophages, B cells and dendritic cells. These immune cells present antigen to helper T cells with CD4 stabilizing the MHC/TCR interaction. The interaction between MHC class II and the TCR ultimately results in an antibody mediated immune response. Other co-stimulatory molecules, such as CD45, CD28 and CD2 aid in T cell activation in the immunological synapse and initiate the formation of the TCR signalosome, a macromolecular protein complex responsible for intracellular signaling.

Several antibodies that bind to human CD3 epsilon are known in the art, e.g. the antibody OKT3 (see e.g. Kung, P. et al., Science 206 (1979) 347-349; Salmeron, A. et al., J Immunol 147 (1991) 3047-3052), the antibody UCHT1 (see e.g. Callard, R.E. et al., Clin Exp Immunol 43 (1981) 497-505) or the antibody SP34 (see e.g. Pessano, S. et al., EMBO J 4 (1985) 337-344). From these, seemingly only the antibody SP34 is human cynomolgus cross-reactive (Conrad M.L., et. al., Cytometry A 71 (2007) 925-933).

WO 2007/042261 reports compositions comprising cross-species-specific antibodies and uses thereof. In Soo Young Yang, et al., LN USA 137 (1986) 1097-1100) a common pathway for T lymphocyte activation involving both the CD3-Ti complex and CD2 sheep erythrocyte receptor determinants are reported. WO 2012/158818 reports multi-specific Fab fusion proteins and methods of use. In DD 272473 a method for the production of monoclonal antibodies against the epsilon chain of the CD3 antigen of human T lymphocytes is reported.

Summary

The disclosure provides a method and its use for producing a human cynomolgus cross-reactive antibody.

It has been found that by immunizing a non-human experimental animal solely with a cynomolgus antigen, i.e. without immunizing the experimental animal before or thereafter with the human homolog, a human cynomolgus cross-reactive antibody can be obtained.

As reported herein is the use of a method comprising the step of immunizing a non-human experimental animal with a native cynomolgus antigen as sole antigen for producing/generating/obtaining a human cynomolgus cross-reactive antibody. One aspect as reported herein is the use of a method comprising the step of immunizing a non-human experimental animal with a native cynomolgus antigen as sole CD3 epsilon antigen for producing a human cynomolgus cross-reactive antibody.

In one embodiment the native cynomolgus antigen lacks one or more (contiguous) amino acid stretches that are present in the corresponding human antigen, whereby one of the lacking (contiguous) amino acid stretches in the corresponding human antigen is the main immunogenic site/epitope of the human antigen. In one embodiment the (native cynomolgus) antigen is CD3 epsilon and the human cynomolgus cross-reactive antibody specifically binds to (native) human CD3 epsilon of SEQ ID NO: 02 and specifically binds to a polypeptide of SEQ ID NO: 01. In one embodiment the non-human experimental animal is immunized one or more times with primary cynomolgus PBLs, whereby the PBLs are (optionally) enriched for T cells. In one embodiment the immunizing comprises as first step (injection) an intradermal application, as second step (injection) an intramuscular application and as third step (injection) a subcutaneous application. In one embodiment the method is without the use of/using a denaturing agent. In one embodiment the human cynomolgus cross-reactive antibody specifically binds to human and cynomolgus CD3 epsilon, to the polypeptide of SEQ ID NO: 01 and activates human T cells.

As reported herein is the use of a method comprising the step of immunizing a non-human experimental animal three times with primary cynomolgus PBLs, (optionally) enriched for T cells, without the use of/using primary human PBLs as immunogen and without the use of/using a denaturing agent for producing/generating/obtaining a human cynomolgus cross-reactive antibody specifically binding to human CD3 epsilon of SEQ ID NO: 02 and specifically binding to a polypeptide of SEQ ID NO: 01, wherein the antibody specifically binds to human and cynomolgus T cells, activates human T cells, and does not bind to the same epitope as the antibody OKT3 (see e.g. Kung, P. et al., Science 206 (1979) 347-349; Salmeron, A. et al., J Immunol 147 (1991) 3047-3052), the antibody UCHT1 (see e.g. Callard, R.E. et al., Clin Exp Immunol 43 (1981) 497-505) or the antibody SP34 (see e.g. Pessano, S. et al., EMBO J 4 (1985) 337-344). Another aspect as reported herein is the use of a method comprising the step of immunizing a non-human experimental animal, three times with primary cynomolgus PBLs, whereby the PBLs are enriched for T cells, without using primary human PBLs as immunogen and without using a denaturing agent for producing a human cynomolgus cross-reactive antibody specifically binding to human CD3 epsilon of SEQ ID NO: 02 and specifically binding to a polypeptide of SEQ ID NO: 01, wherein the human cynomolgus cross-reactive antibody specifically binds to human and cynomolgus T cells, activates human T cells and

does not bind to the same epitope as antibody the OKT3, the antibody UCHT1 and/or the antibody SP34 and/or the antibody reported in WO2007/042261. In one embodiment the antibody as reported herein does not bind to the same epitope as the antibody reported in WO 2007/042261. In one embodiment the antibody as reported herein does not bind to the same epitope as the antibody reported in WO 2007/042261 and specifically binds to human and cynomolgus T cells and(/or) activates human T cells.

Another aspect as reported herein is a method for producing a human cynomolgus cross-reactive antibody comprising the step of immunizing a non-human experimental animal with a native cynomolgus antigen as sole CD3 epsilon antigen.

In one embodiment the native cynomolgus antigen lacks one or more (contiguous) amino acid stretches that are present in the corresponding human antigen, whereby one of the lacking (contiguous) amino acid stretches in the corresponding human antigen is the main immunogenic epitope of the human antigen. In one embodiment the non-human experimental animal is immunized one or more times with primary cynomolgus PBLs, whereby the PBLs are (optionally) enriched for T cells. In one embodiment the immunizing comprises as first step an intradermal application, as second step an intramuscular application and as third step a subcutaneous application.

Another aspect as reported herein is a method for producing a human cynomolgus cross-reactive antibody that specifically binds to human CD3 epsilon of SEQ ID NO: 02 and that specifically binds to a polypeptide of SEQ ID NO: 01 comprising the step of immunizing a non-human experimental animal three times with primary cynomolgus PBLs, whereby the PBLs are enriched for T cells, without using primary human PBLs as immunogen and without using a denaturing agent, wherein the human cynomolgus cross-reactive antibody specifically binds to human and cynomolgus T cells, activates human T cells, and does not bind to the same epitope as the antibody OKT3, the antibody UCHT1 and/or the antibody SP34 and/or the antibody reported in WO2007/042261.

Another aspect as reported herein is a human cynomolgus cross-reactive antibody that specifically binds to human CD3 epsilon of SEQ ID NO: 02 and that specifically binds to a polypeptide of SEQ ID NO: 01, wherein the human cynomolgus cross-reactive antibody specifically binds to human and cynomolgus T cells and activates human T cells and does not bind to the same epitope as the antibody OKT3, the antibody UCHT1 and/or antibody the SP34 and/or the antibody reported in WO 2007/042261. In one embodiment the antibody as reported herein does not bind to the same epitope as the antibody reported in WO 2007/042261. In one embodiment the antibody as reported herein does not bind to the same epitope as the antibody reported in WO 2007/042261 and specifically binds to human and cynomolgus T cells and(/or) activates human T cells.

As reported herein is a human cynomolgus cross-reactive antibody that specifically binds to human CD3 epsilon of SEQ ID NO: 02 and that specifically binds to a polypeptide of SEQ ID NO: 01, wherein the human cynomolgus cross-reactive antibody specifically binds to human and cynomolgus T cells, activates human T cells and does not bind to the same epitope as the antibody OKT3, the antibody UCHT1 and/or the antibody SP34 and/or the antibody reported in WO 2007/042261.

As reported herein is a human cynomolgus cross-reactive antibody that specifically binds to human CD3 epsilon of SEQ ID NO: 02 and that specifically binds to a polypeptide of SEQ ID NO: 01 obtainable/obtained by immunizing a non-human experimental animal three times with primary cynomolgus PBLs, whereby the PBLs are (optionally) enriched for T cells, without the use of/using primary human PBLs as immunogen and without using a denaturing agent, wherein the human cynomolgus cross-reactive antibody specifically binds to human and cynomolgus T cells, activates human T cells, and does not bind to the same epitope as the antibody OKT3, the antibody UCHT1 and/or the antibody SP34. In one embodiment the antibody as reported herein does not bind to the same epitope as the antibody reported in WO 2007/042261. In one embodiment the antibody as reported herein does not bind to the same epitope as the antibody reported in WO 2007/042261 and specifically binds to human and cynomolgus T cells and(/or) activates human T cells.

Detailed Description of the Invention

T cells are key effectors of the adaptive immune response, with a number of important roles in the elimination of pathogens and in autoimmune diseases. There are several subsets of T cells, each with a distinct function.

T helper cells assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. These cells are also known as CD4+ T cells because they express the CD4 glycoprotein on their surface. T helper cells become activated when they are presented with peptide antigens by MHC class II molecules, which are expressed on the surface of APCs. Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response.

Cytotoxic T cells destroy virally infected cells and tumor cells, and are also implicated in transplant rejection. These cells are also known as CD8+ T cells since they express the CD8 glycoprotein at their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of all nucleated cells.

Memory T cells are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells

Patent Claims

1. Use of a method comprising the step of immunizing a non-human experimental animal with a native cynomolgus antigen as sole CD3 epsilon antigen for producing a human cynomolgus cross-reactive antibody.

2. Use according to claim 1 wherein the native cynomolgus antigen lacks one or more (contiguous) amino acid stretches that are present in the corresponding human antigen, whereby one of the lacking (contiguous) amino acid stretches in the corresponding human antigen is the main immunogenic epitope of the human antigen.

3. Use according to claims 1 to 2 wherein the native cynomolgus antigen is CD3 epsilon and the human cynomolgus cross-reactive antibody specifically binds to (native) human CD3 epsilon of SEQ ID NO: 02 and specifically binds to a polypeptide of SEQ ID NO: 01.

4. Use according to claims 1 to 3 wherein the non-human experimental animal is immunized one or more times with primary cynomolgus PBLs (peripheral blood lymphocytes), whereby the PBLs are enriched for T cells.

5. Use according to claims 1 to 4 wherein the immunizing comprises as first step an intradermal application, as second step an intramuscular application and as third step a subcutaneous application.

6. Use according to claims 1 to 5 wherein the method is without using a denaturing agent.

7. Use according to claims 1 to 6 wherein the human cynomolgus cross-reactive antibody specifically binds to human and cynomolgus CD3 epsilon, to the polypeptide of SEQ ID NO: 01 and activates human T cells.

8. Use of a method comprising the step of immunizing a non-human experimental animal, three times with primary cynomolgus PBLs, whereby the PBLs are enriched for T cells, without using primary human PBLs as immunogen and without using a denaturing agent for producing a human cynomolgus cross-reactive antibody specifically binding to human CD3 epsilon of SEQ ID NO: 02 and specifically binding to a polypeptide of SEQ ID NO: 01, wherein the human cynomolgus cross-reactive antibody specifically binds to human and cynomolgus T cells, activates human T cells

and does not bind to the same epitope as antibody the OKT3, the antibody UCHT1 and/or the antibody SP34 and/or the antibody reported in WO2007/042261.

9. Method for producing a human cynomolgus cross-reactive antibody comprising the step of immunizing a non-human experimental animal with a native cynomolgus antigen as sole CD3 epsilon antigen.

10. Method according to claim 9 wherein the native cynomolgus antigen lacks one or more (contiguous) amino acid stretches that are present in the corresponding human antigen, whereby one of the lacking (contiguous) amino acid stretches in the corresponding human antigen is the main immunogenic epitope of the human antigen.

11. Method according to claims 9 or 10 wherein the non-human experimental animal is immunized one or more times with primary cynomolgus PBLs, whereby the PBLs are enriched for T cells.

12. Method according to claims 9 to 11 wherein the immunizing comprises as first step an intradermal application, as second step an intramuscular application and as third step a subcutaneous application.

13. Method for producing a human cynomolgus cross-reactive antibody specifically binding to human CD3 epsilon of SEQ ID NO: 02 and specifically binding to a polypeptide of SEQ ID NO: 01 comprising the step of immunizing a non-human experimental animal, three times with primary cynomolgus PBLs, whereby the PBLs are enriched for T cells without using primary human PBLs as immunogen and without using a denaturing agent, wherein the human cynomolgus cross-reactive antibody specifically binds to human and cynomolgus T cells, activates human T cells and does not bind to the same epitope as the antibody OKT3, the antibody UCHT1 and/or the antibody SP34 and/or the antibody reported in WO2007/042261.

14. Human cynomolgus cross-reactive antibody specifically binding to human CD3 epsilon of SEQ ID NO: 02 and specifically binding to a polypeptide of SEQ ID NO: 01 wherein the human cynomolgus cross-reactive antibody specifically binds to human and cynomolgus T cells and activates human T cells and does not bind to the same epitope as the antibody OKT3, the antibody UCHT1 and/or antibody the SP34 and/or the antibody reported in WO 2007/042261.