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METHOD FOR DETECTING A PHENOTYPE AND A FUNCTION OF A CD141+ DENDRITIC CELL SUBSET AND KIT FOR USE

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ABSTRACT

Provided are an antibody combination and an analysis method for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset and a use thereof. The antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset includes a CD141 antibody, a Linage antibody, an HLA-DR antibody, an IL-10 antibody, an IL-12 antibody, an IL-23 antibody, and an IL-27 antibody. Further provided is a kit for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset. The kit can efficiently and rapidly analyze the phenotype and the function of the $CD141^+$ dendritic cell subset in peripheral blood. The kit has a high accuracy rate and can reduce the economic cost due to the detection of a large number of surface antigen molecules. The detection and analysis method is simple and feasible and has an important application value.

METHOD FOR DETECTING A PHENOTYPE AND A FUNCTION OF A CD141$^+$ DENDRITIC CELL SUBSET AND KIT FOR USE

TECHNICAL FIELD

The present application belongs to the field of biotechnology and, in particular, related to an antibody combination and an analysis method for detecting a phenotype and a function of a CD141$^+$ dendritic cell subset and a use thereof.

BACKGROUND

CD141$^+$ dendritic cells are distributed in human peripheral blood and a dendritic cell subset that has been discovered in recent years. Clinical and basic studies have shown that the CD141$^+$ dendritic cell subset plays an important role in the development of multiple diseases, for example, malignant tumor (e.g., lung cancer, melanoma, prostate cancer, and kidney cancer), dermatitis, viral infection (e.g., HIV-1 infection), infectious diseases (e.g., malaria), and some autoimmune diseases (e.g., rheumatoid arthritis). Clinical data has shown that the CD141$^+$ dendritic cells exhibit phenotypic and functional abnormalities in all these diseases. Therefore, the clinical data of the determined phenotype and function of the CD141$^+$ dendritic cells may become an auxiliary index for clinicians to determine the development conditions and clinical treatment effects of the above diseases and is of great significance in clinical diagnosis.

Flow cytometry analysis technology has been widely used in clinical and scientific researches as the main technology of immunology. Having a primary regulatory effect in the immune system of a human body, dendritic cells are one of the hot spots in immunology research. At present, the dendritic cells are mainly detected depending on the flow cytometry analysis technology, but there are various analysis schemes for a flow cytometer for detecting the dendritic cells, without a unified and standardized mode. With the rapid changes in the studies of dendritic cells, multiple different dendritic cell subtypes have been reported. However, the relatively extensive analysis schemes for the flow cytometer for detecting the dendritic cells cannot meet the clinical requirement for accurate analyses of dendritic cells of different subtypes.

CN105911292A discloses a kit for a combined analysis of CD11c$^+$ and CD11b$^+$ dendritic cell

subsets and degrees of differentiation and functions thereof. The kit includes the following eight antibodies: a CD11c antibody, a CD80 antibody, a CD86 antibody, a CD11b antibody, an HLA-DR antibody, an IL-12 antibody, an IL-23 antibody, and an IL-27 antibody. The kit can detect the $CD11c^+$ and $CD11b^+$ dendritic cell subsets and their degrees of differentiation and functions once for a complete set of data. However, the dendritic cells have different morphologies and immune functions, there are a large number of surface antigen molecules of the dendritic cells, and different specific molecules need to be selected for detecting different dendritic cell subsets.

Studies have shown that the $CD11c^+$ and $CD11b^+$ dendritic cell subsets and the $CD141^+$ dendritic cell subset have completely different functions and play roles in different diseases. Therefore, the kit for analyzing the $CD11c^+$ and $CD11b^+$ dendritic cell subsets and their degrees of differentiation and functions cannot meet the requirement for studying the $CD141^+$ dendritic cell subset. Therefore, it is important to develop an immunoassay kit for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset.

SUMMARY

The present application provides an antibody combination and an analysis method for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset and a use thereof. The antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset detects antigens including CD141, Linage, HLA-DR, IL-10, IL-12, IL-23, and IL-27. The present application further provides a kit for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset. The kit can efficiently and rapidly analyze the phenotype and the function of the $CD141^+$ dendritic cell subset in peripheral blood. The kit has a high accuracy rate and reduces the economic cost due to the detection of a large number of surface antigen molecules. In addition, the detection and analysis method is simple and feasible and has an important application value.

In a first aspect, the present application provides an antibody combination for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset. The antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset includes a CD141 antibody, a Linage antibody, an HLA-DR antibody, an IL-10 antibody, an IL-12 antibody, an IL-23 antibody, and an IL-27 antibody.

In the prior art, to identify a dendritic cell subset through flow cytometry, peripheral blood

mononuclear cells generally need to be isolated and extracted. This process is complex and tedious and requires a long time period. If the cell subset is analyzed by detecting surface antigens of cell molecules, a large number of surface antigen molecules of dendritic cells generally need to be selected for detection, and only then detection accuracy and specificity can be improved. However, the detection and analysis of a large number of surface antigens takes a long time and the detection has a relatively high economic cost, which is not conducive to the rapid and efficient analysis and study of the dendritic cell subset. In the present application, the phenotype and the function of the dendritic cell subset are detected by a combination of seven antibody molecules specifically selected, which includes the CD141 antibody, the Linage antibody, the HLA-DR antibody, the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody. The formulation design can detect the phenotype and function of the $CD141^+$ dendritic cell subset with high specificity and high sensitivity, providing the basis for relevant scientific researches.

Preferably, an antibody in the antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset is labelled with a fluorescent dye.

Preferably, the fluorescent dye includes any one of BV510, BV-786, Pacific blue, PE-Cy7, AF700, eFluor660, or PE or a combination of at least two selected therefrom.

In a second aspect, the present application provides a kit for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset. The kit for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset includes the antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in the first aspect.

A kit commercially available at present for testing dendritic cells can only analyze the overall data of the dendritic cells in general and cannot conduct a functional analysis. With the development of scientific researches, multiple new dendritic cell subsets in human peripheral blood have been discovered, such as $CD141^+$ dendritic cells, where these new dendritic cell subsets have different phenotypes and functions. The kit provided by the present application for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset can analyze a proportion of a $CD141^+$ phenotype in the dendritic cell subset, determine a differentiation and maturation situation of the $CD141^+$ dendritic cell subset, and analyze functions of the $CD141^+$ dendritic cell subset.

In a third aspect, the present application provides an analysis method for detecting a phenotype

and a function of a $CD141^+$ dendritic cell subset in the second aspect. The analysis method includes the following steps:

(1) pre-treating peripheral blood to isolate $CD141^+$ dendritic cells;

(2) incubating and detecting the $CD141^+$ dendritic cells by using a CD141 antibody, a Linage antibody, an HLA-DR antibody, an IL-10 antibody, an IL-12 antibody, an IL-23 antibody, and an IL-27 antibody which are labelled with different fluorescent dyes; and

(3) detecting and analyzing the $CD141^+$ dendritic cells through flow cytometry.

In the present application, the analysis method determines the dendritic cell subset in human peripheral blood and a function thereof by a one-step whole blood method. Compared with the determination of dendritic cells by peripheral blood mononuclear cell (PBMC) isolation technology, the analysis method in the present application is simple, feasible, and cost-effective. The isolation of dendritic cells by traditional PBMC isolation technology requires a large amount (generally dozens of milliliters) of blood to be collected and consumes a long time. However, in the present application, the determination by the one-step whole blood method can obtain the required complete set of information by only one drop (10-100 μL) of blood of a patient. The long time required for isolating PBMCs is saved, and the determination can be achieved simply, rapidly, and with only one step. The analysis method is suitable for testing a large batch of clinical samples.

Preferably, step (1) of pre-treating the peripheral blood to isolate the $CD141^+$ dendritic cells includes:

mixing the peripheral blood with an erythrocyte lysate, placing the mixture still without light, centrifuging the mixture to isolate the $CD141^+$ dendritic cells, resuspending the $CD141^+$ dendritic cells in a cell staining solution, and adding leukocyte-stimulating factors for an incubation.

In the present application, the peripheral blood has a volume of 10-100 μL, which may be, for example, 10 μL, 20 μL, 30 μL, 40 μL, 50 μL, 60 μL, 70 μL, 80 μL, 90 μL, or 100 μL, etc.

In the present application, the peripheral blood and the erythrocyte lysate are mixed at a volume ratio of (1-10):200, which may be, for example, 1:200, 3:200, 5:200, 7:200, 9:200, or 10:200, etc.

Preferably, the mixture is placed still without light for 10-15 min, which may be, for example, 10 min, 11 min, 12 min, 13 min, 14 min, or 15 min, etc. and the mixture is placed still without light at a temperature of 20-25 °C, which may be, for example, 20 °C, 21 °C, 22 °C, 23 °C, 24 °C, or 25 °C, etc.

In the present application, the mixture is centrifuged with a centrifugal force of 300-350 g, which may be, for example, 300 g, 310 g, 320 g, 330 g, 340 g, or 350 g, etc. and the mixture is centrifuged for 5-10 min, which may be, for example, 5 min, 6 min, 7 min, 8 min, 9 min, or 10 min, etc.

Preferably, the cell staining solution includes a PBS buffer containing fetal bovine serum.

Preferably, a final concentration of the leukocyte-stimulating factors in a resuspension of the $CD141^+$ dendritic cells is 0.08-0.1 wt%, which may be, for example, 0.08 wt%, 0.09 wt%, or 0.1 wt%, etc.

Preferably, the incubation is conducted for 4-6 h, which may be, for example, 4 h, 5 h, or 6 h, etc. and the incubation is conducted at a temperature of 35-38 °C, which may be, for example, 35 °C, 36 °C, 37 °C, or 38 °C, etc.

Preferably, step (2) of incubating and detecting the $CD141^+$ dendritic cells by using the CD141 antibody, the Linage antibody, the HLA-DR antibody, the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody which are labelled with the different fluorescent dyes includes:

(a) subjecting the $CD141^+$ dendritic cells to a primary incubation by using the CD141 antibody, the Linage antibody, and the HLA-DR antibody which are labelled with different fluorescent dyes; and

(b) subjecting the $CD141^+$ dendritic cells to a secondary incubation by using the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody which are labelled with different fluorescent dyes.

Preferably, step (a) of subjecting the $CD141^+$ dendritic cells to the primary incubation by using the CD141 antibody, the Linage antibody, and the HLA-DR antibody which are labelled with the different fluorescent dyes includes:

resuspending the $CD141^+$ dendritic cells after the incubation in step (1), subjecting a resuspension of the $CD141^+$ dendritic cells and the CD141 antibody, the Linage antibody, and the HLA-DR antibody which are labelled with the different fluorescent dyes to the primary incubation, immobilizing the $CD141^+$ dendritic cells with a formalin solution, and subjecting the $CD141^+$ dendritic cells to an incubation with light shielded.

Preferably, the formalin solution has a mass fraction of 1.5-2.5 vol%, which may be, for example, 1.5 vol%, 1.8 vol%, 2.0 vol%, 2.2 vol%, or 2.5 vol%, etc.

Preferably, the primary incubation is conducted for 25-35 min, which may be, for example, 25 min, 28 min, 30 min, 32 min, or 35 min, etc. and the primary incubation is conducted at a temperature of 20-25 °C, which may be, for example, 20 °C, 21 °C, 22 °C, 23 °C, 24 °C, or 25 °C, etc.

Preferably, the incubation with light shielded is conducted for 15-20 min, which may be, for example, 15 min, 16 min, 17 min, 18 min, or 20 min, etc. and the incubation with light shielded is conducted at a temperature of 20-25 °C, which may be, for example, 20 °C, 21 °C, 22 °C, 23 °C, 24 °C, or 25 °C, etc.

Preferably, step (b) of subjecting the $CD141^+$ dendritic cells to the secondary incubation by using the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody which are labelled with the different fluorescent dyes includes:

resuspending the $CD141^+$ dendritic cells obtained in step (a) with a cell penetrating solution, centrifuging the cell penetrating solution to remove a supernatant, resuspending a cell penetrating solution for precipitation, and subjecting a resuspension of the $CD141^+$ dendritic cells and the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody which are labelled with the different fluorescent dyes to the secondary incubation.

Preferably, the cell penetrating solution is centrifuged with a centrifugal force of 300-350 g, which may be, for example, 300 g, 310 g, 320 g, 330 g, 340 g, or 350 g, etc. and the cell penetrating solution is centrifuged for 5-10 min, which may be, for example, 5 min, 6 min, 7 min, 8 min, 9 min, or 10 min, etc.

Preferably, the secondary incubation is conducted for 24-26 h, which may be, for example, 24 h, 25 h, or 26 h, etc. and the secondary incubation is conducted at a temperature of 0-4 °C, which

may be, for example, 0 °C, 1 °C, 2 °C, 3 °C, or 4 °C, etc.

Preferably, the secondary incubation is conducted with light shielded.

Preferably, step (3) of detecting and analyzing the $CD141^+$ dendritic cells through the flow cytometry includes:

resuspending the $CD141^+$ dendritic cells after the secondary incubation with a cell penetrating solution, centrifuging the cell penetrating solution to remove a supernatant, resuspending precipitated cells with a cell staining solution, and detecting and analyzing the $CD141^+$ dendritic cells through the flow cytometry.

Preferably, the cell penetrating solution is centrifuged with a centrifugal force of 300-350 g, which may be, for example, 300 g, 310 g, 320 g, 330 g, 340 g, or 350 g, etc. and the cell penetrating solution is centrifuged for 5-10 min, which may be, for example, 5 min, 6 min, 7 min, 8 min, 9 min, or 10 min, etc.

Preferably, a method for analyzing the $CD141^+$ dendritic cells includes:

analyzing a proportion of a $CD141^+$ phenotype in the $CD141^+$ dendritic cell subset according to binding situations of the CD141 antibody and the Linage antibody to CD141 and Linage on surfaces of the $CD141^+$ dendritic cells;

analyzing a differentiation and maturation situation of the $CD141^+$ dendritic cell subset according to a binding situation of the HLA-DR antibody to HLA-DR on the surfaces of the $CD141^+$ dendritic cells; and

analyzing functions of the $CD141^+$ dendritic cell subset according to binding situations of the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody to IL-10, IL-12, IL-23, and IL-27 on the surfaces of the $CD141^+$ dendritic cells.

As a preferred technical solution of the present application, the analysis method for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset includes the steps described below.

(1) Peripheral blood is pre-treated to isolate $CD141^+$ dendritic cells:

mixing 10-100 μL of peripheral blood with an erythrocyte lysate, placing the mixture still without light for 10-15 min at 20-25 °C, centrifuging the mixture for 5-10 min with a centrifugal force of 300-350 g to isolate the $CD141^+$ dendritic cells, resuspending the $CD141^+$ dendritic cells in a cell staining solution, adding leukocyte-stimulating factors, and conducting an incubation for 4-6 h at 35-38 °C, where a final concentration of the leukocyte-stimulating factors in a resuspension of the $CD141^+$ dendritic cells is 0.08-0.1 wt%.

(2) The $CD141^+$ dendritic cells are incubated and detected by using a CD141 antibody, a Linage antibody, an HLA-DR antibody, an IL-10 antibody, an IL-12 antibody, an IL-23 antibody, and an IL-27 antibody which are labelled with different fluorescent dyes:

(a) resuspending the $CD141^+$ dendritic cells after the incubation in step (1), subjecting a resuspension of the $CD141^+$ dendritic cells and the CD141 antibody, the Linage antibody, and the HLA-DR antibody which are labelled with different fluorescent dyes to a primary incubation for 25-35 min at a temperature of 20-25 °C, immobilizing the $CD141^+$ dendritic cells with a formalin solution with a mass fraction of 1.5-2.5 vol%, and subjecting the $CD141^+$ dendritic cells to an incubation with light shielded for 15-20 min at a temperature of 20-25 °C, where a volume ratio of the resuspension of the $CD141^+$ dendritic cells to the CD141 antibody, the Linage antibody, and the HLA-DR antibody is 100:(2-2.5):(2-2.5):(2-2.5); and

(b) resuspending the $CD141^+$ dendritic cells obtained in step (a) with a cell penetrating solution, centrifuging the cell penetrating solution for 5-10 min with a centrifugal force of 300-350 g to remove a supernatant, resuspending a cell penetrating solution for precipitation, and subjecting a resuspension of the $CD141^+$ dendritic cells and the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody which are labelled with different fluorescent dyes to a secondary incubation, where a volume ratio of the resuspension of the $CD141^+$ dendritic cells to the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody is 100:(2-2.5):(2-2.5):(2-2.5): (2-2.5), and the secondary incubation is conducted with light shielded for 24-26 h at a temperature of 0-4 °C.

(3) The $CD141^+$ dendritic cells are detected and analyzed through flow cytometry:

resuspending the $CD141^+$ dendritic cells after the secondary incubation with a cell penetrating solution, centrifuging the cell penetrating solution for 5-10 min with a centrifugal force of 300-350 g to remove a supernatant, resuspending precipitated cells with a cell staining solution, and detecting and analyzing the $CD141^+$ dendritic cells through the flow cytometry;

analyzing a proportion of a $CD141^+$ phenotype in the $CD141^+$ dendritic cell subset according to binding situations of the CD141 antibody and the Linage antibody to CD141 and Linage on surfaces of the $CD141^+$ dendritic cells;

analyzing a differentiation and maturation situation of the $CD141^+$ dendritic cell subset according to a binding situation of the HLA-DR antibody to HLA-DR on the surfaces of the $CD141^+$ dendritic cells; and

analyzing functions of the $CD141^+$ dendritic cell subset according to binding situations of the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody to IL-10, IL-12, IL-23, and IL-27 on the surfaces of the $CD141^+$ dendritic cells.

In a fourth aspect, the present application provides a system for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset. The system for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset includes:

(1) a sample treatment module configured to pre-treat peripheral blood to isolate $CD141^+$ dendritic cells;

(2) a detection module configured to incubate and detect the $CD141^+$ dendritic cells by using a CD141 antibody, a Linage antibody, an HLA-DR antibody, an IL-10 antibody, an IL-12 antibody, an IL-23 antibody, and an IL-27 antibody which are labelled with different fluorescent dyes; and

(3) an analysis module configured to detect and analyze the $CD141^+$ dendritic cells through flow cytometry.

In a fifth aspect, the present application provides a use of any one of the antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in the first aspect, the kit for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in the second aspect, or the system for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in the fourth aspect or a combination of at least two selected therefrom for identifying the phenotype and the function of the $CD141^+$ dendritic cell subset and/or preparing a product for identifying the phenotype and the function of the $CD141^+$ dendritic cell subset.

Preferably, the product includes a kit and/or a detection reagent for detecting malignant tumor.

Preferably, the malignant tumor includes any one of lung cancer, melanoma, prostate cancer, or kidney cancer or a combination of at least two selected therefrom.

Any numerical range described in the present application includes not only the above-listed point values but also any point values within the numerical range which are not listed. Due to the limitation of space and the consideration of simplicity, specific point values included in the range are not exhaustively listed in the present application.

Compared with the prior art, the present application has the beneficial effects described below.

(1) The analysis method for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in the present application is simple, rapid, and feasible. The dendritic cell subset in the human peripheral blood and the function thereof are determined by the one-step whole blood method. Compared with the determination of dendritic cells by peripheral blood mononuclear cell (PBMC) isolation technology, the analysis method in the present application is simple, feasible, and cost-effective. The isolation of dendritic cells by traditional PBMC isolation technology requires a large amount (generally dozens of milliliters) of blood to be collected and consumes a long time. However, in the present application, the determination by the one-step whole blood method can obtain the required complete set of information by only one drop (10-100 μL) of blood of a patient. The long time required for isolating PBMCs is saved, and the determination can be achieved simply, rapidly, and with only one step. The analysis method is suitable for testing a large batch of clinical samples.

(2) The result of the analysis method for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in the present application is comprehensive information. In the present application, seven antibody molecules are specifically selected as a combination which includes the CD141 antibody, the Linage antibody, the HLA-DR antibody, the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody. The formulation design can analyze the proportion of the $CD141^+$ phenotype in the $CD141^+$ dendritic cell subset with high specificity and high sensitivity, determine the differentiation and maturation situation of the $CD141^+$ dendritic cell subset, and analyze the functions of the $CD141^+$ dendritic cell subset.

(3) The result of the analysis method for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in the present application has high accuracy. Compared with other techniques, the analysis scheme for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in the present application has the advantages of high sensitivity and good

specificity and can obtain a more accurate and reliable detection result.

(4) An analysis scheme of the kit commercially available at present for analyzing dendritic cells can only test the overall data of the dendritic cells and cannot conduct a functional analysis. The kit for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in the present application can finely provide a complete set of data for the phenotype and the function of the latest $CD141^+$ dendritic cell subset in the human peripheral blood. Compared with the previously developed schemes, the present application tests the phenotype of the $CD141^+$ dendritic cell subset in combination with the function thereof for the first time.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is an FSC-SSC scatter plot of peripheral blood cells of a healthy person in Example 3.

FIG. 2 is flow cytometry analysis plots of peripheral blood of a healthy person in Example 3.

FIG. 3 is a plot showing flow cytometry analysis results of $CD141^+$ dendritic cell subsets in peripheral blood of a healthy person/non-small-cell lung carcinoma patient in Example 3.

FIG. 4 is a statistical plot of the expression of HLA-DR in $CD141^+$ dendritic cell subsets in peripheral blood of a healthy person/non-small-cell lung carcinoma patient in Example 3.

FIG. 5 is a plot showing the expression proportions of IL-10 on $CD141^+$ dendritic cells in peripheral blood of an isotype control/healthy person/lung carcinoma patient in Example 3.

FIG. 6 is a statistical plot of the expression of IL-10 on $CD141^+$ dendritic cells in peripheral blood of a healthy person/lung carcinoma patient in Example 3.

FIG. 7 is a plot showing the expression proportions of IL-12 on $CD141^+$ dendritic cells in peripheral blood of an isotype control/healthy person/lung carcinoma patient in Example 3.

FIG. 8 is a statistical plot of the expression of IL-12 on $CD141^+$ dendritic cells in peripheral blood of a healthy person/lung carcinoma patient in Example 3.

FIG. 9 is a plot showing the expression proportions of IL-23 on $CD141^+$ dendritic cells in peripheral blood of an isotype control/healthy person/lung carcinoma patient in Example 3.

FIG. 10 is a statistical plot of the expression of IL-23 on $CD141^+$ dendritic cells in peripheral

blood of a healthy person/lung carcinoma patient in Example 3.

FIG. 11 is a plot showing the expression proportions of IL-27 on $CD141^+$ dendritic cells in peripheral blood of an isotype control/healthy person/lung carcinoma patient in Example 3.

FIG. 12 is a statistical plot of the expression of IL-27 on $CD141^+$ dendritic cells in peripheral blood of a healthy person/lung carcinoma patient in Example 3.

DETAILED DESCRIPTION

Technical solutions of the present application are further described below through specific examples. Those skilled in the art are to understand that the examples described herein are used for a better understanding of the present application and are not to be construed as specific limitations to the present application.

Experiments without specific techniques or conditions specified in the examples are conducted according to techniques or conditions described in the literature in the art or product specifications. The reagents or instruments used herein without manufacturers specified are conventional products commercially available from proper channels.

Experimental materials used in the following specific examples:

flow cytometer (Cytek); Linage antibody (BioLegend); CD141 antibody (BioLegend); HLA-DR antibody (eBioscience); IL-10 antibody (BD); IL-12 antibody (BioLegend); IL-23 antibody (eBioscience); and IL-27 antibody (BioLegend).

Example 1

This example provides an antibody combination for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset. The antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset includes a CD141 antibody (BioLegend), a Linage antibody (BioLegend), an HLA-DR antibody (eBioscience), an IL-10 antibody (BD), an IL-12 antibody (BioLegend), an IL-23 antibody (eBioscience), and an IL-27 antibody (BioLegend).

An antibody in the antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset is labelled with a fluorescent dye. The fluorescent dye for the CD141 antibody is BV510; the fluorescent dye for the Linage antibody is BV786; the

fluorescent dye for the HLA-DR antibody is Pacific blue; the fluorescent dye for the IL-10 antibody is PE-Cy7; the fluorescent dye for the IL-12 antibody is AF700; the fluorescent dye for the IL-23 antibody is eFluor660; and the fluorescent dye for the IL-27 antibody is PE.

Example 2

This example provides a kit for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset. The kit for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset includes the antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in Example 1.

Example 3

In this example, the kit for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset in Example 2 was used for analyzing the differentiation and functions of the $CD141^+$ dendritic cell subset in the peripheral blood of a non-small-cell lung carcinoma patient (experimental group) and a healthy person (control group) and analyzing an isotype control.

(1) The peripheral blood was pre-treated to isolate $CD141^+$ dendritic cells.

100 μL of vein peripheral blood of the non-small-cell lung carcinoma patient and 100 μL of vein peripheral blood of the healthy adult were taken separately and anticoagulated. The whole blood of the peripheral blood was mixed with 2 mL of 1× erythrocyte lysate (BioLegend), rotated and shaken for 10s, placed still without light for 15 min at 25 °C, and centrifuged in a centrifuge (for 5 min with 350 g). The supernatant was poured out. The precipitated cells were suspended in 2 mL of cell staining solution (PBS solution containing 2.5% fetal bovine serum), leukocyte-stimulating factors (BD) were added, and the cells were incubated for 6 h at a constant temperature of 37 °C for later use. The final concentration of the leukocyte-stimulating factors in the resuspension of the $CD141^+$ dendritic cells was 0.1%.

(2) The $CD141^+$ dendritic cells were incubated and detected by using a CD141 antibody, a Linage antibody, an HLA-DR antibody, an IL-10 antibody, an IL-12 antibody, an IL-23 antibody, and an IL-27 antibody which were labelled with different fluorescent dyes:

(a) the $CD141^+$ dendritic cells obtained in step (1) were resuspended in 100 μL of cell staining solution, the resuspension of the $CD141^+$ dendritic cells and the CD141 antibody labelled with 2 μL of BV510, the Linage antibody labelled with 2 μL of BV786, and the HLA-DR antibody

labelled with 2 μL of Pacific blue were subjected to a primary incubation for 30 min at 25 °C, 2 mL of cell staining solution was added and centrifuged for 5 min with 350 g twice, and the $CD141^+$ dendritic cells were immobilized with 2 mL of formalin solution with a mass fraction of 2% and incubated with light shielded for 20 min at 25 °C; and

(b) the $CD141^+$ dendritic cells obtained in step (a) were resuspended with 2 mL of cell penetrating solution (BioLegend), centrifuged for 5 min with 350 g twice, and resuspended with 100 μL of cell penetrating solution for precipitation, and the resuspension of the $CD141^+$ dendritic cells and the IL-10 antibody labelled with 2 μL of PE-Cy7, the IL-12 antibody labelled with 2 μL of AF700, the IL-23 antibody labelled with 2 μL of eFluor660, and the IL-27 antibody labelled with 2 μL of PE were subjected to a secondary incubation with light shielded for 30 min at 25 °C.

(3) The $CD141^+$ dendritic cells were detected and analyzed through flow cytometry.

The $CD141^+$ dendritic cells after the secondary incubation were resuspended with 2 mL of cell penetrating solution, centrifuged for 5 min with 350 g to remove the supernatant, and the precipitated cells were resuspended with 0.5 mL of cell staining solution and detected and analyzed through the flow cytometry.

All information about the dendritic cell subset (characterized by the expression of CD141 and Linage), a differentiation degree (characterized by the expression of HLA-DR) and a cell function (characterized by the expression of IL-10, IL-12, IL-23 and IL-27) was obtained once by the above method through the flow cytometry so that the differences between the non-small-cell lung carcinoma patient and the healthy person could be studied through the comparison of these information, thereby providing a basis for studying the immunomodulatory mechanism of the non-small-cell lung carcinoma patient.

Analysis results

(1) The proportion of the $CD141^+$ phenotype of the dendritic cells was analyzed according to the binding situations of the CD141 antibody and the Linage (Lin) antibody to CD141 and Linage on the surfaces of the $CD141^+$ dendritic cells. The detection results are shown in FIG. 1. FIG. 1 is an FSC-SSC scatter plot of peripheral blood cells of the healthy person, where FSC denotes the size of the cells and SSC denotes the granularity of the cells. FIG. 2 is flow cytometry analysis plots of the peripheral blood of the healthy person, where the left plot shows

the expression proportion of Linage on the dendritic cells in the peripheral blood of the healthy person, Linage$^-$ (Lin$^-$) accounts for 12.9% of the total number of cells, the right plot shows the expression proportion of CD141 on the dendritic cells in the peripheral blood of the healthy person, and CD141$^+$ accounts for 41.9% of the total number of cells.

(2) The expression of a co-stimulatory molecule HLA-DR on the CD141$^+$ dendritic cell subset in human peripheral blood was detected by flow cytometer, and the data was used for assessing the differentiation and maturation situation of the CD141$^+$ dendritic cell subset in the human peripheral blood. FIG. 3 is a plot showing flow cytometry analysis results of the CD141$^+$ dendritic cell subsets in the peripheral blood of the healthy person/non-small-cell lung carcinoma patient. FIG. 4 is a statistical plot of the expression of HLA-DR in the CD141$^+$ dendritic cell subsets in the peripheral blood of the healthy person/non-small-cell lung carcinoma patient. In FIG. 3, a thick line represents the healthy person and a dashed line represents the non-small-cell lung carcinoma patient. The results show that the higher the HLA-DR co-expression on the surfaces of the CD141$^+$ dendritic cells, the higher the immune maturity of the CD141$^+$ dendritic cells. It can also be seen from the results in FIGS. 3 and 4 that the HLA-DR co-expression on the surfaces of the CD141$^+$ dendritic cells of the healthy person is significantly higher than that of the lung carcinoma patient, indicating that the immune maturity of the CD141$^+$ dendritic cells in the lung carcinoma patient is significantly lower than that in the healthy person.

(3) The functions of the CD141$^+$ dendritic cell subset were analyzed by determining the secretory expression of IL-10, IL-12, IL-23, and IL-27 on the surfaces of the CD141$^+$ dendritic cells through the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody.

FIG. 5 is a plot showing the expression proportions of IL-10 on the CD141$^+$ dendritic cells in the peripheral blood of the isotype control/healthy person/lung carcinoma patient. FIG. 6 is a statistical plot of the expression of IL-10 on the CD141$^+$ dendritic cells in the peripheral blood of the healthy person/lung carcinoma patient. IL-10 has the effect of inhibiting an *in vivo* immune function. It can be seen from the results in FIGS. 5 and 6 that the CD141$^+$ dendritic cells of the non-small-cell lung carcinoma patient secrete more IL-10 than the CD141$^+$ dendritic cells of the healthy person. The results indicate that the *in vivo* CD141$^+$ dendritic cells of the non-small-cell lung carcinoma patient inhibit an *in vivo* anti-tumor immune response by secreting more IL-10.

FIG. 7 is a plot showing the expression proportions of IL-12 on the CD141$^+$ dendritic cells in

the peripheral blood of the isotype control/healthy person/lung carcinoma patient. FIG. 8 is a statistical plot of the expression of IL-12 on the $CD141^+$ dendritic cells in the peripheral blood of the healthy person/lung carcinoma patient. IL-12 is a cytokine that promotes an immune response. It can be seen from the results in FIGS. 7 and 8 that the *in vivo* $CD141^+$ dendritic cells of the non-small-cell lung carcinoma patient secrete less amount of IL-12 than the $CD141^+$ dendritic cells of the healthy person. The results indicate that the *in vivo* $CD141^+$ dendritic cells of the non-small-cell lung carcinoma patient secrete insufficient IL-12, which is very likely to affect an IL-12-mediated anti-tumor immune response.

FIG. 9 is a plot showing the expression proportions of IL-23 on the $CD141^+$ dendritic cells in the peripheral blood of the isotype control/healthy person/lung carcinoma patient. FIG. 10 is a statistical plot of the expression of IL-23 on the $CD141^+$ dendritic cells in the peripheral blood of the healthy person/lung carcinoma patient. FIG. 11 is a plot showing the expression proportions of IL-27 on the $CD141^+$ dendritic cells in the peripheral blood of the isotype control/healthy person/lung carcinoma patient. FIG. 12 is a statistical plot of the expression of IL-27 on the $CD141^+$ dendritic cells in the peripheral blood of the healthy person/lung carcinoma patient. It can be seen from the figures that the expression proportions of IL-23 and IL-27 on the $CD141^+$ dendritic cells of the healthy person are 17.7% and 36.6%, respectively; and the expression proportions of IL-23 and IL-27 on the $CD141^+$ dendritic cells of the non-small-cell lung carcinoma patient are 22.2% and 38.8%, respectively.

IL-23 is a cytokine that promotes an immune response. If the dendritic cells can secrete more IL-23, it indicates that the dendritic cells can promote the immune response by secreting more amount of IL-23. The results in FIGS. 9 and 10 show that IL-23 secreted by the $CD141^+$ dendritic cells of the normal healthy person is similar to IL-23 secreted by the $CD141^+$ dendritic cells of the lung carcinoma patient, indicating that the *in vivo* $CD141^+$ dendritic cells of the lung carcinoma patient do not affect a $CD141^+$DC-mediated immune response by regulating IL-23.

IL-27 is a cytokine that can inhibit an immune function. If the dendritic cells secrete more IL-27, it indicates that the dendritic cells have an immunosuppression effect and can inhibit an immune response by secreting more amount of IL-27. The detection results in FIGS. 11 and 12 show that the amount of IL-27 secreted by the *in vivo* $CD141^+$ dendritic cells of the lung carcinoma patient is not significantly different from the amount of IL-27 secreted by the $CD141^+$ dendritic cells of the normal healthy person, indicating that the *in vivo* $CD141^+$ dendritic cells of the lung carcinoma patient do not inhibit the immune function by secreting more amount of IL-27. The *in vivo* $CD141^+$ dendritic cells of the lung carcinoma patient have an immunosuppression

function compared with the *in vivo* $CD141^+$ dendritic cells of the healthy person.

The above results indicate that the combination of antibody molecules and the analysis method of the present application can effectively identify the $CD141^+$ dendritic cell subset in the peripheral blood and analyze the differentiation and maturation situation and functions thereof.

To conclude, in the present application, seven antibody molecules are specifically selected as a combination which includes the CD141 antibody, the Linage antibody, the HLA-DR antibody, the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody. The formulation design can analyze the proportion of the $CD141^+$ phenotype in the $CD141^+$ dendritic cell subset with high specificity and high sensitivity, determine the differentiation and maturation situation of the $CD141^+$ dendritic cell subset, and analyze the functions of the $CD141^+$ dendritic cell subset.

The applicant states that the above are the specific examples of the present application and not intended to limit the protection scope of the present application. Those skilled in the art should understand that any changes or substitutions easily conceivable by those skilled in the art within the technical scope disclosed in the present application fall within the protection scope and the disclosed scope of the present application.

What is claimed is:

1. An antibody combination for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset, comprising a CD141 antibody, a Linage antibody, an HLA-DR antibody, an IL-10 antibody, an IL-12 antibody, an IL-23 antibody, and an IL-27 antibody.

2. The antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset according to claim 1, wherein an antibody in the antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset is labelled with a fluorescent dye.

3. The antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset according to claim 2, wherein the fluorescent dye comprises any one of BV510, BV-786, Pacific blue, PE-Cy7, AF700, eFluor660, or PE or a combination of at least two selected therefrom.

4. A kit for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset, comprising the antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset according to any one of claims 1 to 3.

5. An analysis method for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset, comprising the following steps:

(1) pre-treating peripheral blood to isolate $CD141^+$ dendritic cells;

(2) incubating and detecting the $CD141^+$ dendritic cells by using a CD141 antibody, a Linage antibody, an HLA-DR antibody, an IL-10 antibody, an IL-12 antibody, an IL-23 antibody, and an IL-27 antibody which are labelled with different fluorescent dyes; and

(3) detecting and analyzing the $CD141^+$ dendritic cells through flow cytometry.

6. The analysis method for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset according to claim 5, wherein step (1) of pre-treating the peripheral blood to isolate the $CD141^+$ dendritic cells comprises:

mixing the peripheral blood with an erythrocyte lysate, placing the mixture still without light, centrifuging the mixture to isolate the $CD141^+$ dendritic cells, resuspending the $CD141^+$ dendritic cells in a cell staining solution, and adding leukocyte-stimulating factors for an incubation;

preferably, the mixture is placed still without light for 10-15 min at a temperature of 20-25 °C;

preferably, the cell staining solution comprises a PBS buffer containing fetal bovine serum;

preferably, a final concentration of the leukocyte-stimulating factors in a resuspension of the $CD141^+$ dendritic cells is 0.08-0.1 wt%;

preferably, the incubation is conducted for 4-6 h at a temperature of 35-38 °C.

7. The analysis method for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset according to claim 5 or 6, wherein step (2) of incubating and detecting the $CD141^+$ dendritic cells by using the CD141 antibody, the Linage antibody, the HLA-DR antibody, the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody which are labelled with the different fluorescent dyes comprises:

(a) subjecting the $CD141^+$ dendritic cells to a primary incubation by using the CD141 antibody, the Linage antibody, and the HLA-DR antibody which are labelled with different fluorescent dyes; and

(b) subjecting the $CD141^+$ dendritic cells to a secondary incubation by using the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody which are labelled with different fluorescent dyes.

8. The analysis method for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset according to claim 7, wherein step (a) of subjecting the $CD141^+$ dendritic cells to the primary incubation by using the CD141 antibody, the Linage antibody, and the HLA-DR antibody which are labelled with the different fluorescent dyes comprises:

resuspending the $CD141^+$ dendritic cells after the incubation in step (1), subjecting a resuspension of the $CD141^+$ dendritic cells and the CD141 antibody, the Linage antibody, and the HLA-DR antibody which are labelled with the different fluorescent dyes to the primary incubation, immobilizing the $CD141^+$ dendritic cells with a formalin solution, and subjecting the $CD141^+$ dendritic cells to an incubation with light shielded;

preferably, the formalin solution has a mass fraction of 1.5-2.5 vol%;

preferably, the primary incubation is conducted for 25-35 min at a temperature of 20-25 °C;

preferably, the incubation with light shielded is conducted for 15-20 min at a temperature of 20-25 °C;

preferably, step (b) of subjecting the $CD141^+$ dendritic cells to the secondary incubation by using the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody which

are labelled with the different fluorescent dyes comprises:

resuspending the $CD141^+$ dendritic cells obtained in step (a) with a cell penetrating solution, centrifuging the cell penetrating solution to remove a supernatant, resuspending a cell penetrating solution for precipitation, and subjecting a resuspension of the $CD141^+$ dendritic cells and the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody which are labelled with the different fluorescent dyes to the secondary incubation;

preferably, the cell penetrating solution is centrifuged for 5-10 min with a centrifugal force of 300-350 g;

preferably, the secondary incubation is conducted for 24-26 h at a temperature of 0-4 °C;

preferably, the secondary incubation is conducted with light shielded.

9. The analysis method for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset according to any one of claims 5 to 8, wherein step (3) of detecting and analyzing the $CD141^+$ dendritic cells through the flow cytometry comprises:

resuspending the $CD141^+$ dendritic cells after the secondary incubation with a cell penetrating solution, centrifuging the cell penetrating solution to remove a supernatant, resuspending precipitated cells with a cell staining solution, and detecting and analyzing the $CD141^+$ dendritic cells through the flow cytometry;

preferably, the cell penetrating solution is centrifuged for 5-10 min with a centrifugal force of 300-350 g;

preferably, a method for analyzing the $CD141^+$ dendritic cells comprises:

analyzing a proportion of a $CD141^+$ phenotype in the $CD141^+$ dendritic cell subset according to binding situations of the CD141 antibody and the Linage antibody to CD141 and Linage on surfaces of the $CD141^+$ dendritic cells;

analyzing a differentiation and maturation situation of the $CD141^+$ dendritic cell subset according to a binding situation of the HLA-DR antibody to HLA-DR on the surfaces of the $CD141^+$ dendritic cells; and

analyzing functions of the $CD141^+$ dendritic cell subset according to binding situations of the IL-10 antibody, the IL-12 antibody, the IL-23 antibody, and the IL-27 antibody to IL-10, IL-12, IL-23, and IL-27 on the surfaces of the $CD141^+$ dendritic cells.

10. A system for detecting a phenotype and a function of a $CD141^+$ dendritic cell subset, comprising:

(1) a sample treatment module configured to pre-treat peripheral blood to isolate $CD141^+$ dendritic cells;

(2) a detection module configured to incubate and detect the $CD141^+$ dendritic cells by using a CD141 antibody, a Linage antibody, an HLA-DR antibody, an IL-10 antibody, an IL-12 antibody, an IL-23 antibody, and an IL-27 antibody which are labelled with different fluorescent dyes; and

(3) an analysis module configured to detect and analyze the $CD141^+$ dendritic cells through flow cytometry.

11. A use of any one of the antibody combination for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset according to any one of claims 1 to 3, the kit for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset according to claim 4, or the system for detecting the phenotype and the function of the $CD141^+$ dendritic cell subset according to claim 10 or a combination of at least two selected therefrom for identifying the phenotype and the function of the $CD141^+$ dendritic cell subset and/or preparing a product for identifying the phenotype and the function of the $CD141^+$ dendritic cell subset;

preferably, the product comprises a kit and/or a detection reagent for detecting malignant tumor;

preferably, the malignant tumor comprises any one of lung cancer, melanoma, prostate cancer, or kidney cancer or a combination of at least two selected therefrom.

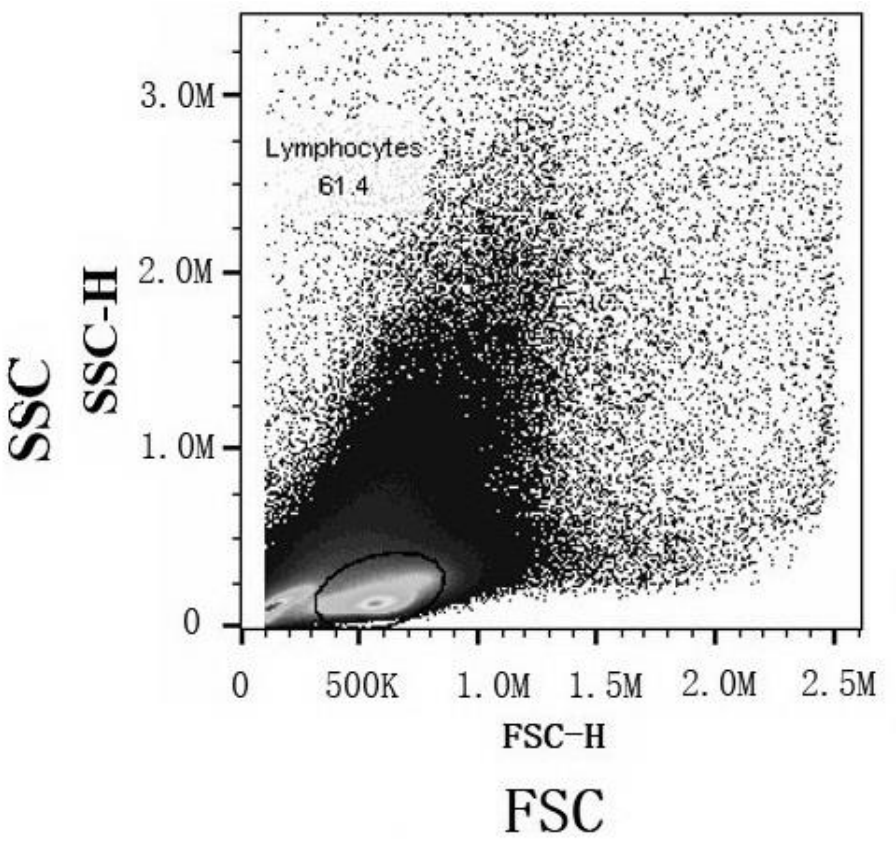
3.0M
2.0M
1.0M
0
SSC
SSC-H
Lymphocytes
61.4
0
500K
1.0M
1.5M
2.0M
2.5M
FSC-H
FSC

FIG.1

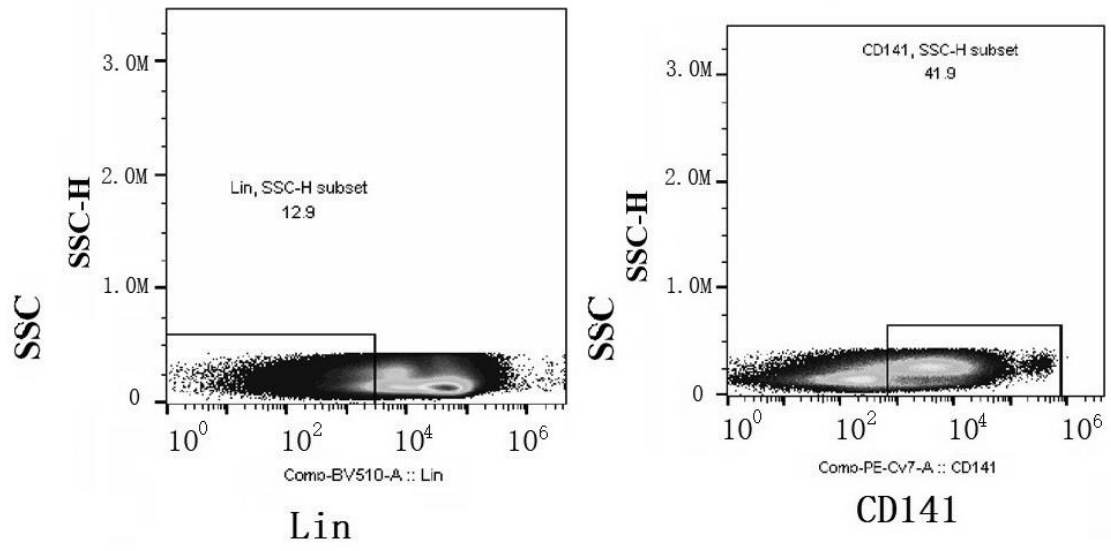
3.0M
2.0M
1.0M
0
SSC
SSC-H
Lin, SSC-H subset
12.9
10^0
10^2
10^4
10^6
Comp-BV510-A :: Lin
Lin
CD141, SSC-H subset
41.9
Comp-PE-Cy7-A :: CD141
CD141

FIG. 2

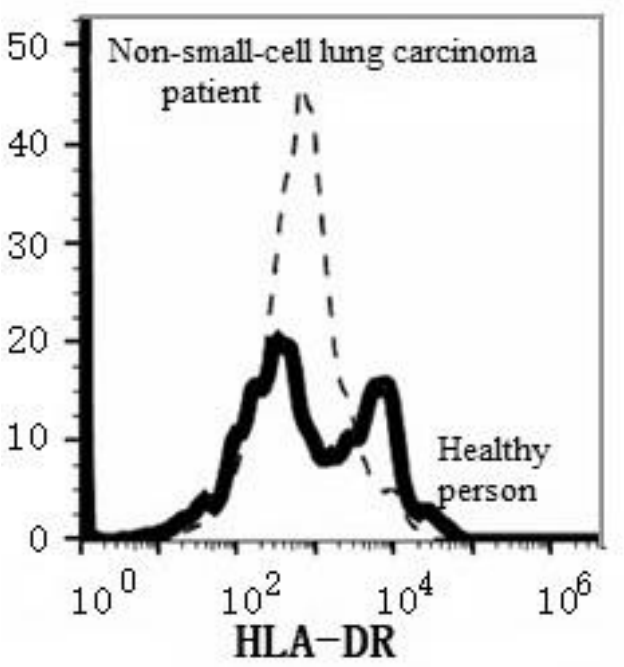
Non-small-cell lung carcinoma patient
Healthy person
50
40
30
20
10
0
10^0
10^2
10^4
10^6
HLA−DR

FIG. 3

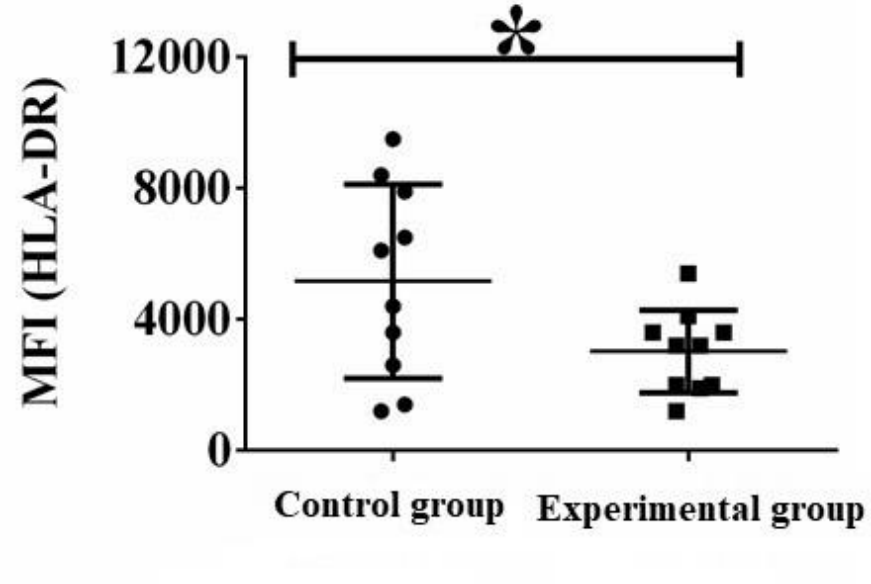
MFI (HLA-DR)
12000
8000
4000
0
*
Control group
Experimental group

FIG. 4

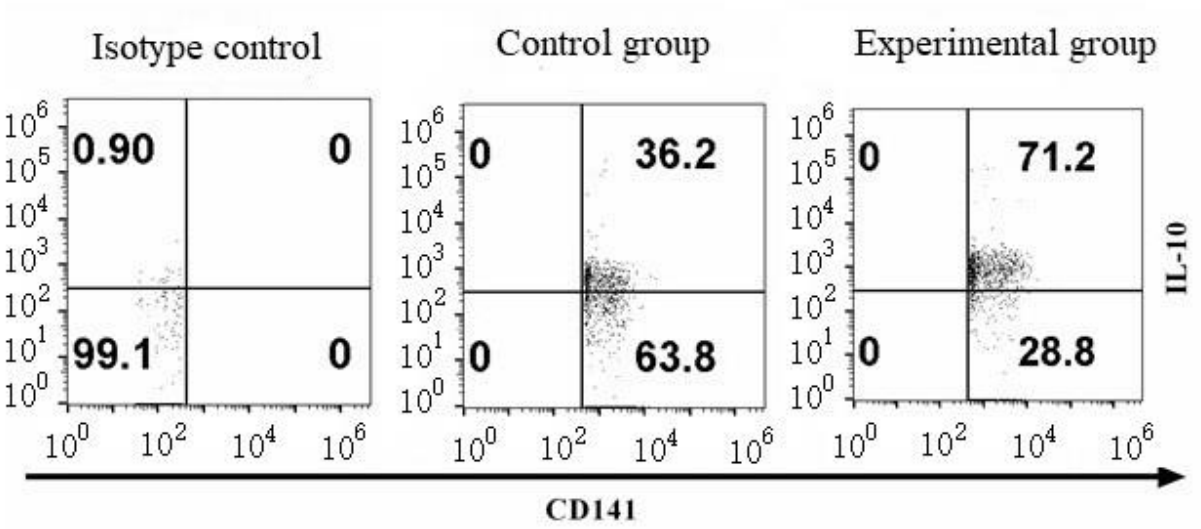
Isotype control
Control group
Experimental group
0.90
0
99.1
0
0
36.2
0
63.8
0
71.2
0
28.8
IL-10
CD141

FIG. 5

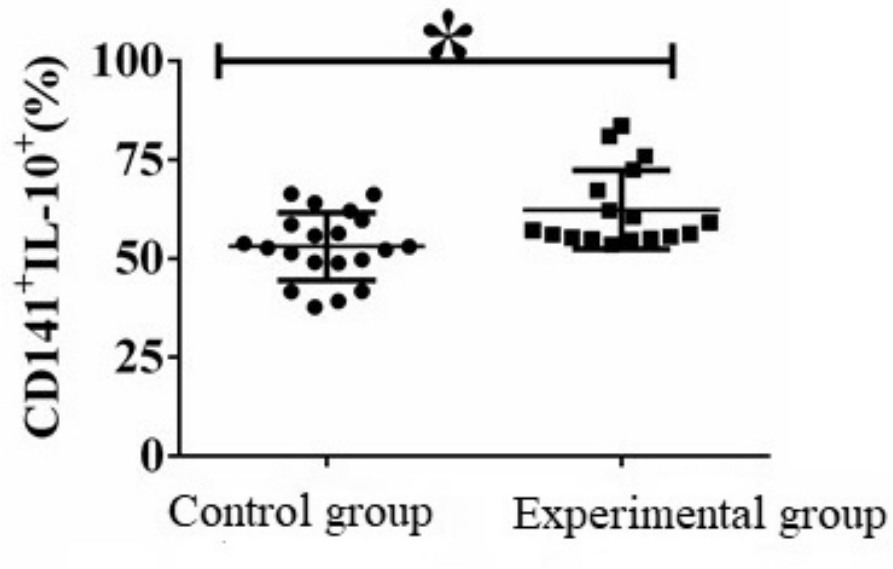
CD141+IL-10+(%)
100
75
50
25
0
Control group
Experimental group

FIG. 6

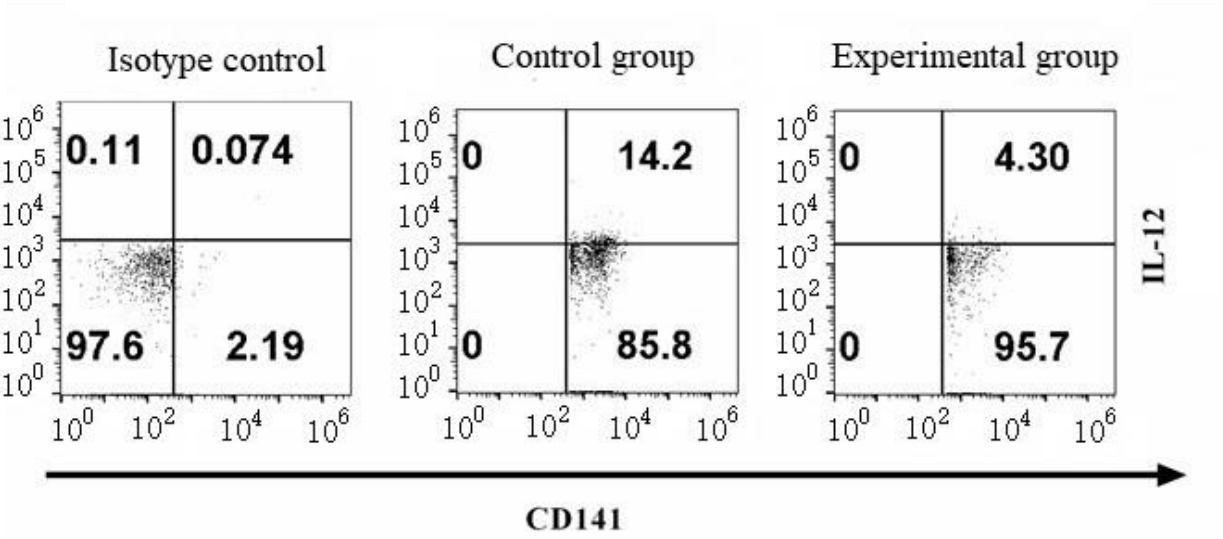
Isotype control
Control group
Experimental group
0.11
0.074
97.6
2.19
0
14.2
0
85.8
0
4.30
0
95.7
IL-12
CD141

FIG. 7

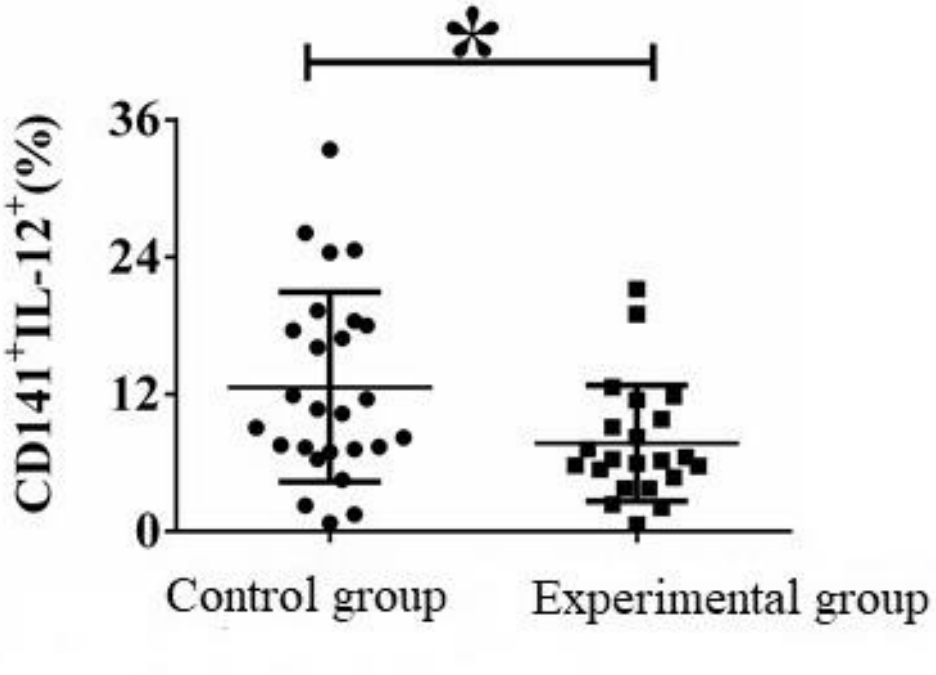
*
36
24
12
0
CD141+IL-12+(%)
Control group
Experimental group

FIG. 8

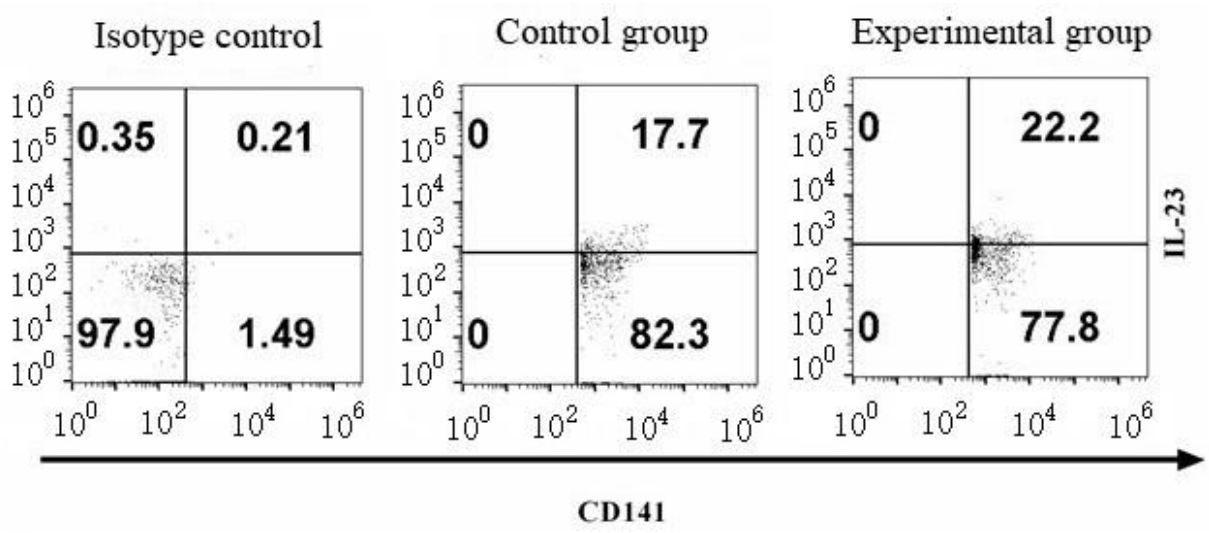
Isotype control
Control group
Experimental group
0.35
0.21
97.9
1.49
0
17.7
0
82.3
0
22.2
0
77.8
IL-23
CD141

FIG. 9

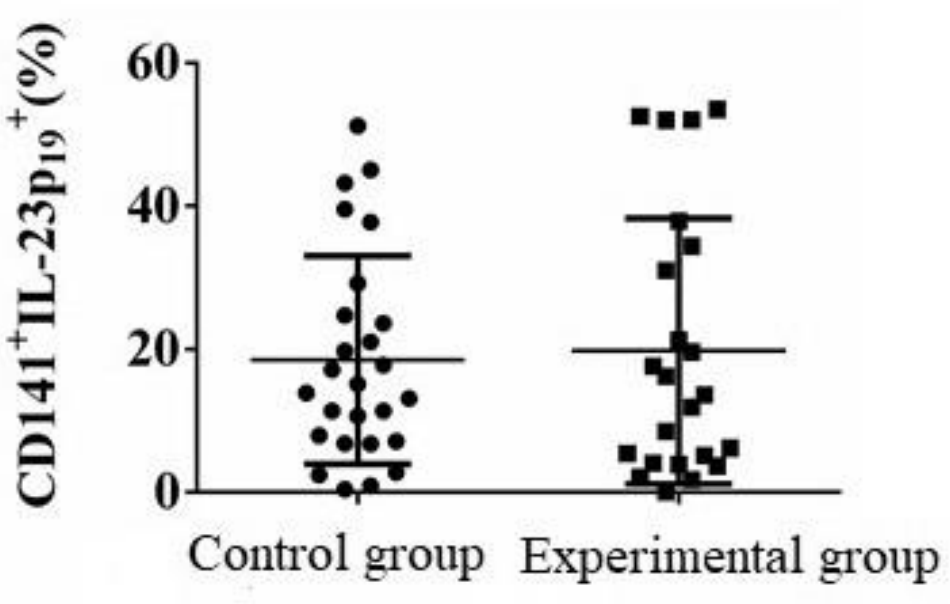
CD141+IL-23p19+(%)
60
40
20
0
Control group
Experimental group

FIG. 10

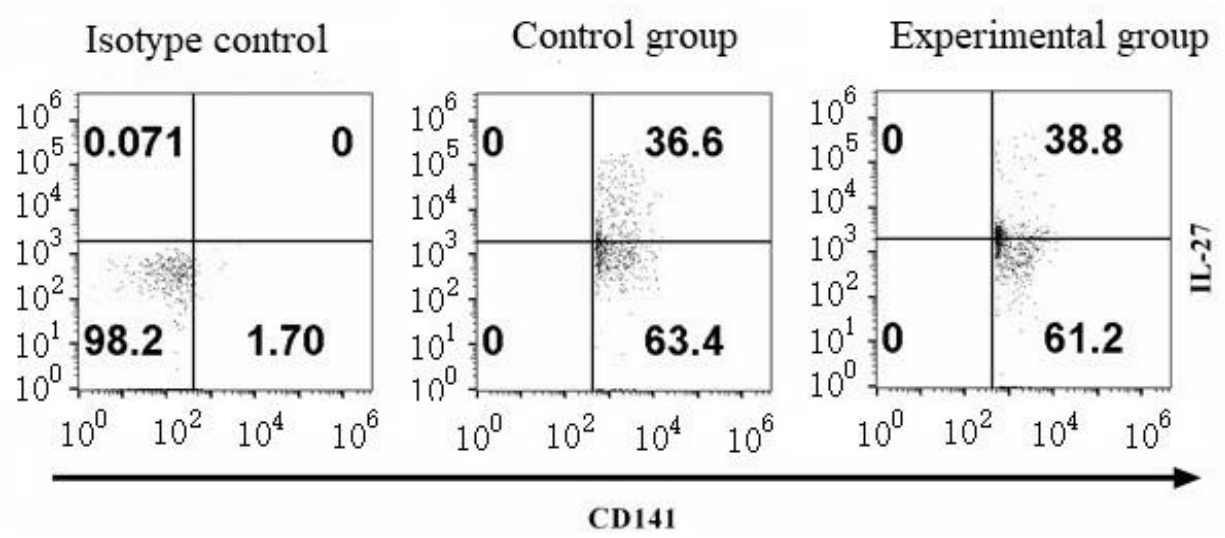
Isotype control
Control group
Experimental group
0.071
0
98.2
1.70
0
36.6
0
63.4
0
38.8
0
61.2
IL-27
CD141

FIG. 11

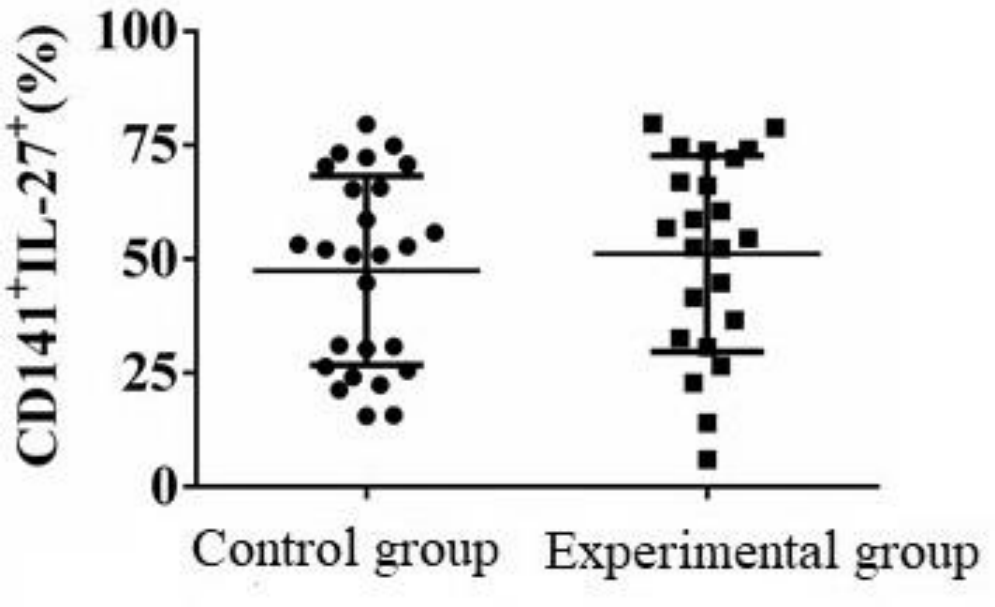
CD141+IL-27+(%)
100
75
50
25
0
Control group
Experimental group

FIG. 12