

DESCRIPTION

Title of the Invention: EYE DISEASE TREATMENT AGENT, SCREENING
METHOD THEREFOR, AND METHOD FOR PREDICTING REJECTION RESPONSE
ASSOCIATED WITH RETINAL PIGMENT EPITHELIAL CELL TRANSPLANT

5 [Technical Field]

[0001]

The present invention relates to an agent for treating
ophthalmic diseases, a screening method therefor and a method
for predicting a rejection response associated with retinal
10 pigment epithelial cell transplantation.

[Background Art]

[0002]

Major histocompatibility complex (MHC) is known as HLA
(human leukocyte antigen) in human and is expressed in almost
15 all cells and tissues. HLA mainly contains 6 gene loci
antigens of A, B, C, DR, DQ and DP, each of which is
constituted of complicated combinations of several dozen kinds
of different types (allele) in tens of thousands of
combinations. HLA is responsible for an important immune
20 mechanism in the human body and the main role thereof is
presentation of antigen to recognize self or non-self. When a
person receives transplantation of a cell or tissue derived
from other person (allogeneic transplantation), HLA is
recognized as the most important antigen (foreign substance) by
25 immunocompetent cells such as T cells and the like, whereby
rejection is formed and the transplant is not engrafted.

[0003]

In normal organ transplantation, particularly bone marrow
transplantation, it is considered that all 6 gene loci of
30 respective antigens A, B, C, DR, DQ and DP of HLA should in
principle match; when they do not match, a risk of severe
rejection response and bad prognosis after transplantation are
foreseeable.

[0004]

35 It is known that retinal pigment epithelial cell

expresses or produces a molecule suppressing inflammatory cells that infiltrate intraocularly (Th1 cell, CD8 positive cell, macrophage, B cell and the like), and affords a suppressive signal on allogeneic autologous inflammatory cells (e.g., non-patent documents 1, 2). On the other hand, it is known that retinal pigment epithelial cell affords a promotive signal on regulatory T cells under similar conditions (non-patent documents 3, 4). On the other hand, retinal pigment epithelial cell is also known to suppress xenogeneic T cells (Th22 cell) (non-patent document 5), and it is known that retinal pigment epithelial cell itself has an immunosuppressive function, due to which the environment around retinal pigment epithelial cell tends to suppress immune reaction. Also, it is known that coculture of a human retinal pigment epithelial cell pre-treated with IFN- γ or macrophage with allogeneic T cells activates T cells since production of IL-2 and the like increases (non-patent document 6). IFN- γ is also known to play an important role in the expression of MHC class II molecule (non-patent document 7). However, an allogeneic transplantation strategy in consideration of HLA compatibility has not existed to date in the transplantation to retina disease patients by using a retinal pigment epithelial cell (hereinafter to be indicated as "RPE cell").

[Document List]

[non-patent documents]

[0005]

non-patent document 1: Sugita S et al, J Immunol. 2004, 172: 4184-4194.

non-patent document 2: Sugita S et al, J Immunol. 2006, 176: 118-127.

non-patent document 3: Sugita S et al, J Immunol, 2008, 181: 7525-7536.

non-patent document 4: Sugita S et al, J Immunol, 2009, 183: 5013-5022.

non-patent document 5: Sugita S et al, IOVS, 2013, vol. 54, No.

10: 6926-6933.

non-patent document 6: Enzmann V et al. Graefes Arch Clin Exp
Ophthalmol. 2001 Jul; 239(6):445-51

non-patent document 7: Enzmann V. Transplant Immunology 7:9-14,
5 1999

[SUMMARY OF THE INVENTION]

[Problems to be Solved by the Invention]

[0006]

An object of the present invention is to provide an agent
10 for treating ophthalmic diseases and a screening method for an
agent for treating ophthalmic diseases and the like. Another
object of the present invention is to provide a method for
predicting immunorejection response associated with
transplantation of retinal pigment epithelial cell in patients
15 with ophthalmic diseases.

[Means of Solving the Problems]

[0007]

The present inventors obtained T cells from plural test
subjects and iPS cell-derived retinal pigment epithelial (RPE)
20 cells, performed HLA typing and then contact culture of the
aforementioned T cells and RPE cells, and measured the
concentration of cytokine produced from the T cells to find
that, when the alleles of 3 gene loci of HLA-A, HLA-B and HLA-
DR of the aforementioned T cells match with the alleles at
25 respective 3 gene loci of HLA-A, HLA-B and HLA-DR of a retinal
pigment epithelial cell wherein the genotype of the 3 gene loci
is each homozygous, the cytokine concentration is equal to or
lower than that of the T cell alone.

The present inventors have conducted further studies
30 based on these findings and completed the present invention.

[0008]

Accordingly, the present invention relates to the
following.

[1] A therapeutic agent for ophthalmic diseases, comprising a
35 retinal pigment epithelial cell having an HLA-A gene locus, an

HLA-B gene locus and an HLA-DR gene locus, wherein each locus has a homozygous genotype, which agent is for a patient with an ophthalmic disease and having a combination wherein at least one of the alleles at each corresponding gene locus is the same
5 as an allele at the gene locus of the retinal pigment epithelial cell.

[2] The agent of [1], which suppresses an immunorejection response after transplantation.

[3] The agent of [1] or [2], wherein the retinal pigment
10 epithelial cell is derived from a pluripotent stem cell.

[4] The agent of [3], wherein the pluripotent stem cell is an iPS cell.

[5] The agent of any one of [1] - [4], wherein the ophthalmic disease is retina denaturation or a disease associated with
15 retina denaturation.

[6] A method of selecting a therapeutic agent for an ophthalmic disease for a patient with the ophthalmic disease, comprising
(i) a step of examining genotypes of HLA-A gene locus, HLA-B gene locus and HLA-DR gene locus of patient-derived T cells,
20 (ii) a step of selecting a retinal pigment epithelial cell having an allele matching the allele at each gene locus clarified in step (i).

[7] The method of [6], wherein the retinal pigment epithelial cell in step (ii) has an HLA-A gene locus, an HLA-B gene locus
25 and an HLA-DR gene locus, and a genotype of each locus is homozygous.

[8] A method of selecting a therapeutic agent for ophthalmic diseases, which is for a patient with an ophthalmic disease, comprising

30 (1) a step of performing contact culture of patient-derived T cells with retinal pigment epithelial cells,
(2) a step of measuring a concentration of inflammatory cytokine secreted in the culture supernatant in step (1), and
(3) a step of judging that the retinal pigment epithelial cell
35 can be used as an active ingredient of a therapeutic agent for

ophthalmic diseases of a patient, when the inflammatory cytokine concentration in step (2) is of the same level as or not more than a concentration of an inflammatory cytokine in a culture supernatant obtained by culturing the patient-derived T
5 cells alone.

[9] The method of [8], wherein the retinal pigment epithelial cell has an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, and a genotype of each locus is homozygous.

[10] The method of [8] or [9], wherein the patient-derived T
10 cells in step (1) are activated using an anti-CD3 antibody.

[11] The method of any one of [8] - [10], wherein the patient-derived T cells in step (1) are CD4 positive, and the inflammatory cytokine measured in step (2) is IFN- γ .

[12] The method of any one of [8] - [10], wherein the patient-
15 derived T cells in step (1) are CD8 positive, and the inflammatory cytokine measured in step (2) is Granzyme B.

[13] A test method for predicting a rejection response in an ophthalmic disease patient to be transplanted with a retinal pigment epithelial cell for transplantation, comprising
20 (A) a step of performing contact culture of patient-derived T cells with said retinal pigment epithelial cells,
(B) a step of measuring a concentration of inflammatory cytokine secreted in the culture supernatant in step (A), and
(C) a step of judging that a rejection response to the retinal
25 pigment epithelial cell occurs, when the inflammatory cytokine concentration in step (B) is significantly higher than a concentration of an inflammatory cytokine in a culture supernatant obtained by culturing the patient-derived T cells alone.

[14] The method of [13], wherein the retinal pigment epithelial cell for transplantation has an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, and a genotype of each locus is homozygous.

[15] The method of [13] or [14], wherein the patient-derived T
35 cells in step (A) are activated using an anti-CD3 antibody.

[16] The method of any one of [13] - [15], wherein the patient-derived T cells in step (A) are CD4 positive, and the inflammatory cytokine measured in step (B) is IFN- γ .

5 [17] The method of any one of [13] - [15], wherein the patient-derived T cells in step (A) are CD8 positive, and the inflammatory cytokine measured in step (B) is Granzyme B.

[18] A method of treating an ophthalmic disease, comprising forming a sheet of a retinal pigment epithelial cell having an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus,
10 wherein each locus has a homozygous genotype, and transplanting the sheet to a patient with an ophthalmic disease and having a combination wherein at least one of the alleles at each corresponding gene locus is the same as an allele at the gene locus of the retinal pigment epithelial cell.

15 [19] The method of [18], wherein the retinal pigment epithelial cell is derived from a pluripotent stem cell.

[20] The method of [19], wherein the pluripotent stem cell is an iPS cell.

[21] The method of any one of [18] - [20], wherein the
20 ophthalmic disease is retina denaturation or a disease associated with retina denaturation.

[22] A retinal pigment epithelial cell having an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, wherein each locus has a homozygous genotype, which cell is for use for
25 the treatment of an ophthalmic disease of a patient with an ophthalmic disease and having a combination wherein at least one of the alleles at each corresponding gene locus is the same as an allele at the gene locus of the retinal pigment epithelial cell.

30 [23] The retinal pigment epithelial cell of [22], wherein the retinal pigment epithelial cell is derived from a pluripotent stem cell.

[24] The retinal pigment epithelial cell of [23], wherein the pluripotent stem cell is an iPS cell.

35 [25] The retinal pigment epithelial cell of any one of [22] -

[24], wherein the ophthalmic disease is retina denaturation or a disease associated with retina denaturation.

[26] A retinal pigment epithelial cell having an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, wherein
5 each locus has a homozygous genotype, which cell is for use for the production of a therapeutic agent for an ophthalmic disease of a patient with an ophthalmic disease and having a combination wherein at least one of the alleles at each
10 corresponding gene locus is the same as an allele at the gene locus of the retinal pigment epithelial cell.

[27] The retinal pigment epithelial cell of [26], wherein the retinal pigment epithelial cell is derived from a pluripotent stem cell.

[28] The retinal pigment epithelial cell of [27], wherein the
15 pluripotent stem cell is an iPS cell.

[29] The retinal pigment epithelial cell of any one of [26] - [28], wherein the ophthalmic disease is retina denaturation or a disease associated with retina denaturation.

[Effect of the Invention]

20 [0009]

In conventional allogeneic transplantation, the HLA type of a donor and that of a recipient are requested to match as far as possible. Particularly, in bone marrow transplantation, it is considered necessary to match all alleles of 6 gene loci
25 antigens known as HLA type. According to the present invention, however, when the genotype of 3 gene loci of HLA-A, HLA-B and HLA-DR on the donor side is homozygous in RPE cell transplantation, rejection response can be suppressed by merely matching one of the HLA loci of the recipient. Since
30 immunologically incompatible cells for transplantation can be distinguished and selected by previously collecting blood samples from the patient, the burden on patients can be reduced by avoiding incompatible cells for transplantation. RPE cells selected by this method can be used for transplantation.

35 [0010]

In addition, the present invention used to determine whether an immunosuppressant is necessary can contribute to the selection of a treatment plan. Thus, the present invention contributes to marked improvement of treatment efficiency by allogeneic transplantation, and also affords effects such as reduction of the preparation costs of cells unsuitable for transplantation and the like.

RPE cells wherein the genotype of 3 gene loci of HLA-A, HLA-B and HLA-DR is homozygous can be used as a therapeutic agent for ophthalmic diseases for patients having a combination wherein at least one of the alleles at the 3 gene loci matches with the allele of the retinal pigment epithelial cell.

[Brief Description of the Drawings]

[0011]

Fig. 1 shows the concentration of IFN- γ in a culture supernatant when CD4 positive T cell suspension (TLHD-1, TLHD-2) and 454E2-iPS-derived RPE cells were cocultured.

Fig. 2 shows the concentration of IFN- γ in a culture supernatant when CD4 positive T cell suspension (TLHD-2, TLHD-3, TLHD-4, TLHD-6, TLHD-7, TLHD-8, TLHD-9) and 454E2-iPS-derived RPE cells were cocultured, and when TLHD-1-derived T cell suspension and RPE cells derived from iPS cell derived from TLHD-1 were cocultured.

Fig. 3 shows the concentration of IFN- γ in a culture supernatant when CD4 positive T cell suspension (TLHD-1) and various RPE cells (No.1 - 3, 6 - 10 in Table 2) or mouse fibroblast were cocultured.

Fig. 4 shows the concentration of IFN- γ in a culture supernatant when CD4 positive T cell suspension (TLHD-1, TLHD-8, TLHD-14, TLHD-21) and 453F2-iPS-derived RPE cells were cocultured.

Fig. 5 shows the concentration of Granzyme B in a culture supernatant when CD8 positive T cell suspension (TLHD-1, TLHD-8, TLHD-14, TLHD-22) and 453F2-iPS-derived RPE cells were cocultured.

Fig. 6 shows the ratio of CD4 positive T cells that produced IL-2, IFN- γ , IL-17, TNF- α , IL-22 by FACS when CD4 positive T cell suspension (TLHD-1) and 454E2-iPS-derived RPE cells were cocultured.

5 Fig. 7 shows the production amount of various cytokines when CD4 positive T cell suspension (TLHD-1, TLHD-10) and 454E2-iPS-derived RPE cells were cocultured.

Fig. 8 shows the ratio of CD8 positive T cells that produced Granzyme B, Perforin and TNF- α by FACS when CD8
10 positive T cell suspension (TLHD-1) and 454E2-iPS-derived RPE cells were cocultured.

Fig. 9 shows the concentration of Granzyme B in a culture supernatant when CD8 positive T cell suspension (TLHD-1, TLHD-3) and 454E2-iPS-derived RPE cells were cocultured.

15 Fig. 10 shows the concentration of Granzyme B in a culture supernatant when CD8 positive T cell suspension (TLHD-2, 4, 5, 8, 10, 12, 13, 14, 16, 17, 18, 19, 20) and 454E2-iPS-derived RPE cells were cocultured.

[Description of Embodiments]

20 [0012]

The present invention provides a therapeutic agent for ophthalmic diseases, comprising a retinal pigment epithelial cell having an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, wherein each locus has a homozygous genotype,
25 which agent is for a patient with an ophthalmic disease and having a combination wherein at least one of the alleles at each corresponding gene locus is the same as an allele at the gene locus of the retinal pigment epithelial cell (hereinafter to be referred to as "the therapeutic agent of the present
30 invention").

[0013]

The therapeutic agent of the present invention contains a retinal pigment epithelial cell. The "retinal pigment epithelial cell" in the present invention refers to an
35 epithelial cell constituting the retinal pigment epithelium,

and a progenitor cell thereof. Whether a retinal pigment epithelial cell or not can be confirmed by, for example, expression of cell markers (RPE65, CRALBP, MERTK, BEST1 etc.), cell forms (intracellular melanin dye deposition, polygonal and flat cell form, formation of polygonal actin bundle etc.) and the like. The progenitor cell of retinal pigment epithelial cell means a cell directed to be induced to differentiate into retinal pigment epithelial cell, and whether a progenitor cell or not can be confirmed by expression of cell markers (Mitf, Pax6, Rx, Crx etc.) and the like. Functional evaluation of retinal pigment epithelial cell can be confirmed using, for example, secretability of cytokine (VEGF, PEDF etc.), phagocytic capacity or the like as an index. These functional evaluation and confirmation operations can be performed by those of ordinary skill in the art by setting appropriate conditions.

[0014]

As the retinal pigment epithelial cell in the present invention, retinal pigment epithelial cells derived from a mammal can be used. Examples of the mammal include mouse, rat, guinea pig, hamster, rabbit, cat, dog, sheep, swine, bovine, horse, goat, monkey and human. Human retinal pigment epithelial cell is preferably used when retinal pigment epithelial cells are produced for the purpose of transplanting to human, and the like.

[0015]

The aforementioned "retinal pigment epithelial cell" may be a primary cell collected directly, or a cell after passage for several generations. The primary retinal pigment epithelial cell can be isolated by a known method and, for example, retinal pigment epithelial cell can be obtained by isolating an eyeball from a dead body, quickly dividing the eyeball at the equator part, removing vitreous body and retina, treating them as necessary with collagenase, hyaluronidase etc., and recovering the cells by abrasion with a cell scraper or

detaching them from the Bruch's membrane with trypsin or EDTA solution, after which the cells are stood in a culture medium, induced to adhere and grow on a culture dish to achieve the growth thereof in a necessary amount, which is followed by
5 appropriate passage by a trypsin treatment and the like to ensure the cell number.

[0016]

Furthermore, the aforementioned "retinal pigment epithelial cell" may be a cell obtained by differentiation
10 induction of a stem cell including somatic stem cells such as pluripotent stem cell, neural stem cell and the like, or a progenitor cell including neural progenitor cell, retina progenitor cell, and a cell obtained by differentiation induction of a pluripotent stem cell is preferable.

15 [0017]

The "pluripotent stem cell" in the present invention means a stem cell having self-replication competence and differentiation pluripotency and is not particularly limited. For example, embryonic stem cells (ES cells), iPS cells
20 (induced pluripotent stem cells) and the like are widely utilized. Preferably, human ES cell or human iPS cell is utilized, and a human iPS cell is more preferably utilized.

[0018]

The "iPS cell" in the present invention means a cell that
25 artificially acquired self-replication competence and differentiation pluripotency by contacting a nuclear reprogramming factor with a somatic cell (e.g., fibroblast, skin cell, lymphocyte etc.). iPS cell was found for the first time by a method including introduction of a nuclear
30 reprogramming factor consisting of Oct3/4, Sox2, Klf4 and c-Myc into a somatic cell (e.g., fibroblast, skin cell etc.) (Cell, 126: p. 663-676, 2006). Thereafter, many researchers are working on various improvements in the combination of reprogramming factors and introduction method of factors, and a
35 variety of production methods of induced pluripotent stem cell

have been reported. However, the production method of iPS cell in the present invention is not particularly limited.

[0019]

In the "retinal pigment epithelial cell" in the present invention, the genotype of HLA-A gene locus, HLA-B gene locus and HLA-DR gene locus is preferably each homozygous. The genotype being homozygous means that two alleles are genes having the same base sequence. In the below-mentioned Examples, retinal pigment epithelial cells obtained by differentiation induction of T cells and iPS cells collected from a test subject were cocultured and it was found that T cells having a combination wherein at least one of the alleles at each corresponding gene locus is the same as an allele at the gene locus of the retinal pigment epithelial cell having an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, wherein each locus has a homozygous genotype, shows the same level of cytokine production as the control (T cells alone), or surprisingly suppressed cytokine production.

[0020]

Conventionally, it is known that retinal pigment epithelial cell expresses a molecule suppressing inflammatory cells including T cells, and affords a promotive signal on regulatory T cells. However, many of these are the results on allogeneic autologous cells (e.g., non-patent documents 1 - 4 in Background Art). On the other hand, the present inventors have reported that retinal pigment epithelial cells suppress xenogeneic T cells (e.g., non-patent document 5 in Background Art); however, this simply shows the function of retinal pigment epithelial cell itself. In fact, in xenogeneic transplantation, an acute rejection response poses a huge problem and, since retinal pigment epithelial cell does not present an antigen, these reports by the present inventors do not suggest the present invention at all.

[0021]

From the foregoing, when the genotype of 3 gene loci of

HLA-A, HLA-B and HLA-DR on the donor side is homozygous in retinal pigment epithelial cell transplantation, rejection response can be suppressed by merely matching one allele of the HLA gene loci of the recipient, and cells for transplantation
5 can be rapidly supplied to ophthalmic disease patients in need of retinal pigment epithelial transplantation. The present invention is an invention completed based on the finding that when the genotype of 3 gene loci of HLA-A, HLA-B and HLA-DR on the donor side is homozygous in allogeneic retinal pigment
10 epithelial cell transplantation, rejection response can be suppressed by merely matching one of the HLA loci of the recipient, and the finding is highly significant. The final object of the present invention is to suppress immunorejection response that may occur in allogeneic transplantation of
15 retinal pigment epithelial cells as much as possible. From such aspects, a retinal pigment epithelial cell having an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, wherein each locus has a homozygous genotype, which is the active ingredient of a therapeutic agent for ophthalmic
20 diseases to be administered to ophthalmic disease patients in the present invention is an allogeneic cell, and a retinal pigment epithelial cell suitable for the patient is selected from various retinal pigment epithelial cells wherein a genotype of the gene locus is homozygous.

25 [0022]

The genotype of HLA-A gene locus, HLA-B gene locus and HLA-DR gene locus of T cells obtained from a patient with an ophthalmic disease can be examined by a known method and, for example, as shown in the Examples below, and can be easily
30 determined by performing HLA typing. In addition, the genotype at each gene locus of the retinal pigment epithelial cell to be used for the agent of the present invention can also be examined by a similar method. A use method is desirable in which various retinal pigment epithelial cells whose genotype
35 at each gene locus is homozygous and whose specific allele has

previously been clarified are stocked, and a cell appropriate for a patient with an ophthalmic disease is selected and used therefrom.

[0023]

5 Examples of the ophthalmic diseases to which the therapeutic agent of the present invention can be applied include retina denaturation and diseases associated with retina denaturation. Examples of the retina denaturation and diseases associated with retina denaturation include retina denaturation
10 diseases such as age-related macular degeneration, retinitis pigmentosa, diabetic retinopathy, retinal detachment and the like.

[0024]

 The ophthalmic disease patients to whom the therapeutic
15 agent of the present invention can be applied are preferably ophthalmic disease patients having a combination wherein at least one of the alleles at each corresponding gene locus is the same as an allele which is homozygous at the HLA-A gene locus, HLA-B gene locus and HLA-DR gene locus of the retinal
20 pigment epithelial cell.

[0025]

 The range of ophthalmic disease site to which the therapeutic agent of the present invention can be applied is appropriately determined according to the target disease,
25 animal species, age, sex, body weight, symptom of the subject of administration, and the like.

[0026]

 As a use method of the therapeutic agent of the present invention, it can be transplanted in a sheet form. A method of
30 forming a sheet of a retinal pigment epithelial cell is known and, for example, is described in WO 2012/115244. A retinal pigment epithelial cell sheet may be transplanted at once or several times by portions. The number of transplantations to be applied is determined according to the disease and according
35 to the medical workers and guideline. For example, when the

disease is age-related macular degeneration, a cell sheet for transplantation may be transplanted twice or more according to the severity. When transplantation is to be performed plural times, the interval is not particularly limited and a period of
5 several days - several weeks may be provided. Alternatively, the therapeutic agent of the present invention with the cells dispersed therein may be directly transplanted to the target site. The number of transplantation to be applied is determined according to the guideline and the like, similar to
10 the case of sheet, and the transplantation period and the like are also determined as appropriate, similar to the case of sheet.

[0027]

The present invention also provides a method of selecting
15 a therapeutic agent for ophthalmic diseases for ophthalmic disease patients.

Specifically, the method of selecting a therapeutic agent for ophthalmic diseases of the present invention (hereinafter to be referred to as "the method (1) of the present invention")
20 includes the following steps:

(i) a step of examining genotypes of HLA-A gene locus, HLA-B gene locus and HLA-DR gene locus of patient-derived T cells,
(ii) a step of selecting a retinal pigment epithelial cell having an allele matching the allele at each gene locus
25 clarified in step (i).

[0028]

In the method (1) of the present invention, step (i), the genotype of HLA-A gene locus, HLA-B gene locus and HLA-DR gene locus of T cells obtained from a patient with an ophthalmic
30 disease can be examined by a known method and, for example, as shown in the Examples below, and can be easily determined by performing HLA typing. The ophthalmic disease of the a patient from which T cells in the method (1) of the present invention derived may be the same as the ophthalmic diseases to be the
35 application target of the therapeutic agent of the present

invention.

[0029]

The retinal pigment epithelial cell having an allele matching the allele at each gene locus clarified in step (i),
5 which is selected in the method (1) of the present invention, step (ii), is useful as a therapeutic agent for ophthalmic diseases that does not cause rejection response on application to ophthalmic disease patients. In the method (1) of the present invention, genotypes of HLA-A gene locus, HLA-B gene
10 locus and HLA-DR gene locus of the retinal pigment epithelial cell are each preferably homozygous. The retinal pigment epithelial cell in the method (1) of the present invention may be prepared in the same manner as in the preparation of the retinal pigment epithelial cell to be contained in the
15 therapeutic agent of the present invention.

[0030]

The present invention also provides a method of selecting a therapeutic agent for ophthalmic diseases for other ophthalmic disease patients.

20 Specifically, the method of selecting a therapeutic agent for ophthalmic diseases of the present invention (hereinafter to be referred to as "the method (2) of the present invention") includes the following steps:

- (1) a step of performing contact culture of patient-derived T
25 cells with retinal pigment epithelial cells,
- (2) a step of measuring a concentration of inflammatory cytokine secreted in the culture supernatant in step (1), and
- (3) a step of judging that the retinal pigment epithelial cell can be used as an active ingredient of a therapeutic agent for
30 ophthalmic diseases of a patient, when the inflammatory cytokine concentration in step (2) is of the same level as or not more than a concentration of an inflammatory cytokine in a culture supernatant obtained by culturing the patient-derived T cells alone.

35 [0031]

In the method (2) of the present invention, step (1), the contact culture of the patient-derived T cells and retinal pigment epithelial cell can be performed according to the method described in the below-mentioned Examples. Specifically, 5 the patient-derived T cells are seeded in a 96 well plate, retinal pigment epithelial cells are added and the mixture is cultured. In this step, the patient-derived T cells may be activated in advance. While an activating method of T cells is not particularly limited, for example, an anti-CD3 antibody may 10 be added. T cells may be prepared by separating to CD4 positive cells and CD8 positive cells in advance.

The retinal pigment epithelial cells to be used in this step may be radiation-treated in advance to avoid growth of retinal pigment epithelial cell in the culture. Furthermore, 15 genotypes of HLA-A gene locus, HLA-B gene locus and HLA-DR gene locus of the retinal pigment epithelial cell to be used for the culture are each preferably homozygous.

[0032]

A culture medium can be used without any particular 20 limitation as long as it is a medium for cell culture which is generally used in the pertinent field. For example, basal media such as F-10 medium, F12 medium, MEM, BME medium, DMEM, α MEM, IMD medium, ES medium, DM-160 medium, Fisher medium, WE medium and RPMI1640 medium and the like can be used. 25 Furthermore, serum (fetal bovine serum etc.), various growth factors (EGF, FGF, HGF, PDGF and the like), antibiotic, amino acid and the like may be added to the basal medium as necessary. The pH of the medium is preferably about 6 - about 8. The culture can be performed, for example, generally at about 30 - 30 about 40°C for about 15 - about 60 hr, preferably 48 hr. For the culture, the ratio of the patient-derived T cells and the retinal pigment epithelial cells is generally 500:1 - 10:1, preferably 400:1 - 25:1, more preferably 200:1 - 50:1. By setting the ratio of the cell number of the patient-derived T 35 cells and retinal pigment epithelial cells within this range, T

cells secrete an appropriate amount of inflammatory cytokine or cell injury substance, which enables more definite determination of whether the retinal pigment epithelial cell to be the target can be applied to the patients.

5 The ophthalmic disease of a patient to be the derivation of the T cells in the method (2) of the present invention may be the same as the ophthalmic disease to be the application target of the therapeutic agent of the present invention, and the retinal pigment epithelial cell may be prepared in the same
10 manner as the retinal pigment epithelial cell to be contained in the therapeutic agent of the present invention.

[0033]

 In step (2) of the method (2) of the present invention, the concentration of inflammatory cytokine secreted in the
15 culture supernatant can be measured according to the method described in the below-mentioned Examples. To be specific, it can be measured by ELISA, Western blot, FACS and the like. The inflammatory cytokine to be measured in this step is not particularly limited as long as it is involved in the rejection
20 response. For example, when CD4 positive cell is used as the patient-derived T cell in step (1), IFN- γ , IL-17 and TNF- α can be mentioned, of which IFN- γ is more preferable. When CD8 positive cell is used as the patient-derived T cell in step (1), Granzyme B, Perforin and TNF- α can be mentioned, of which
25 Granzyme B is more preferable.

[0034]

 As shown in the below-mentioned Examples, retinal pigment epithelial cells obtained by differentiation induction of T cells and iPS cells collected from a test subject were
30 cocultured, and it was found that T cells having a combination wherein at least one of the alleles at each corresponding gene locus is the same as an allele at said gene locus of the retinal pigment epithelial cell having an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, wherein each locus
35 has a homozygous genotype, shows the same level of cytokine

production as the control, or suppressed cytokine production. Therefore, in step (3) of the method (2) of the present invention, it can be judged that the retinal pigment epithelial cell can be used as an active ingredient of a therapeutic agent for ophthalmic diseases of a patient, when the inflammatory cytokine concentration measured in step (2) is of the same level as or not more than a concentration of an inflammatory cytokine in a culture supernatant obtained by culturing the patient-derived T cells alone.

10 [0035]

The present invention also provides a test method for predicting a rejection response in an ophthalmic disease patient to be transplanted with a retinal pigment epithelial cell for transplantation. Specifically, the test method for predicting a rejection response of the present invention (hereinafter to be referred to as "the method (3) of the present invention") includes the following steps:

- (A) a step of performing contact culture of patient-derived T cells with said retinal pigment epithelial cells,
- 20 (B) a step of measuring a concentration of inflammatory cytokine secreted in the culture supernatant in step (A), and
- (C) a step of judging that a rejection response to the retinal pigment epithelial cell occurs, when the inflammatory cytokine concentration in step (B) is significantly higher than a concentration of an inflammatory cytokine in a culture supernatant obtained by culturing the patient-derived T cells alone.

[0036]

Steps (A) and (B) of the method (3) of the present invention can be performed similarly to steps (1) and (2) of the method (2) of the present invention. As shown in the below-mentioned Examples, retinal pigment epithelial cells obtained by differentiation induction of T cells and iPS cells collected from a test subject were cocultured, and it was found that T cells having a combination wherein at least one of the

alleles at each corresponding gene locus is not the same as an allele at said gene locus of the retinal pigment epithelial cell having an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, wherein each locus has a homozygous genotype, 5 shows higher cytokine production than the control. Therefore, in step (C) of the method (3) of the present invention, it can be judged that a rejection response to the retinal pigment epithelial cell occurs, when the inflammatory cytokine concentration in step (B) is significantly higher than a 10 concentration of an inflammatory cytokine in a culture supernatant obtained by culturing the patient-derived T cells alone.

[Examples]

[0037]

15 The present invention is explained in more detail in the following by referring to Examples, which are mere exemplifications and do not limit the scope of the present invention in any way.

[0038]

20 (Preparation Example 1) Preparation of human iPS cell-derived RPE cells

Using iPS cell line 454E2 or 453F2 (provided by Kyoto University) disclosed in Nature Methods 8, 409-412 2011 as iPS cells, and according to of the differentiation induction method 25 described in J Cell Sci (2009)122: 3169-3179, 454E2-iPS cell-derived RPE cells and 453F2-iPS cell-derived RPE cells (both cells have homozygous genotype at HLA-A, -B, -DR3 gene loci) were prepared.

30 In addition, using, as iPS cell line, iPS cells established from human skin-derived fibroblast of a test subject name TLHD-1 according to the method described in Nature (2011) 474: 225-229 instead of 454E2 or 453F2, TLHD-1-iPS cell-derived RPE cells were prepared by a similar method.

[0039]

35 (Preparation Example 2) Preparation of human T cell suspension

CD4 positive T cells and CD8 positive T cells were separated from human peripheral blood (test subject names; TLHD-1 - TLHD-22) by using a MACS magnetic cell sorter, suspended in 100U/ml rhIL-2-containing RPMI culture medium
5 (RPMI 1640 445 ml, FBS 50 ml, penicillin-streptomycin 5 ml) to prepare each cell suspension.

[0040]

(Reference 1) HLA typing

The results of HLA typing of the cells used in Examples 1
10 - 8 by a conventional method are shown in the following Table. The results of HLA typing of respective T cells of test subjects obtained in Preparation Example 2 are shown in Tables 1-1, 1-2, and the results of HLA typing of the human iPS-derived RPE cells of Preparation Example 1 and those induced
15 from other iPS cells are shown in Table 2.

[0041]

Relative to 454E2-iPS cell-derived RPE cells (homozygous at 3 gene loci of HLA-A, HLA-B, HLA-DR), the test subject names TLHD-10 and TLHD-21 had the same alleles in the genotype of 3
20 gene loci of HLA-A, HLA-B, HLA-DR, the test subject names TLHD-6, 18 had the same alleles in the genotype of 2 gene loci of HLA-B, HLA-DR, the test subject names TLHD-2, 5, 7, 9, 11, 12, 13, 14, 15, 17, 20, 22 had the same alleles only in the genotype of 1 gene locus of HLA-A, and the test subject name
25 TLHD-19 had the same alleles only in the genotype of 1 gene locus of HLA-DR.

Relative to 453F2-iPS cell-derived RPE cells (homozygous at 3 gene loci of HLA-A, HLA-B, HLA-DR), the test subject names TLHD-1, 4, 18 had the same alleles only in the genotype of 2
30 gene loci, and the test subject name TLHD-6 had the same alleles only in the genotype of 1 gene locus of HLA-A.

[0042]

[Table 1-1]

No.	T cells	Origin	HLA-A	HLA-B	HLA-DRB1	T cell response to	
						454E2*	HLA-matched to 454E2
1	TLHD1	PBMCs	A11/- (11:01/-)	B62/B67 (15:01/67:01)	DR9/DR16 (09:01/16:02)	CD4 (+) / CD8 (+)	
2	TLHD2	PBMCs	A24/33 (24:02/33:03)	B44/B55 (44:03/55:02)	DR4/DR13 (04:05/13:02)	CD4 (+) / CD8 (-)	A24:02
3	TLHD3	PBMCs	A2/A26 (02:01/26:03)	B44/B61 (40:06/44:03)	DR4/DR8 (04:05/08:03)	CD4 (+) / CD8 (+)	DQB1 06:01
4	TLHD4	PBMCs	A2/A3 (02:01/03:01)	B62/B51 (15:01/51:01)	DR4/DR4 (04:02/04:06)	CD4 (+) / CD8 (+)	
5	TLHD5	PBMCs	A24/A33 (24:02/33:03)	B7/B44 (07:02/44:03)	DR1/DR13 (01:01/13:02)	CD4 (+) / CD8 (+)	A24:02
6	TLHD6	PBMCs	A11/A26 (11:01/26:02)	B39/B52 (39:01/52:01)	DR8/DR15 (08:03/15:02)	CD4 (-) / CD8, nt	B52:01 DRB1 15:02
7	TLHD7	PBMCs	A24/A26 (24:02/26:01)	B35/B54 (35:01/54:01)	DR14/DR15 (14:05/15:01)	CD4 (+) / CD8, nt	A24:02
8	TLHD8	PBMCs	A26/A26 (26:01/26:03)	B13/B35 (13:01/35:01)	DR12/DR15 (12:02/15:01)	CD4 (+) / CD8 (+)	
9	TLHD9	PBMCs	A24/A26 (24:02/26:03)	B51/B54 (51:01/54:01)	DR4/DR12 (04:05/12:01)	CD4 (+) / CD8, nt	A24:02
10	TLHD10	PBMCs	A24/A31 (24:02/31:01)	B61/B52 (40:02/52:01)	DR15/DR15 (15:01/15:02)	CD4 (-) / CD8 (-)	A24:02/B52:01 DRB1 15:02
11	TLHD11	PBMCs	A24/- (24:02/-)	B7/B59 (07:02/59:01)	DR4/DR9 (04:05/09:01)	CD4, nt / CD8 (-)	A24:02
12	TLHD12	PBMCs	A24/A26 (24:02/26:01)	B51/B54 (51:01/54:01)	DR14/DR14 (14:03/14:05)	CD4, nt / CD8 (-)	A24:02
13	TLHD13	PBMCs	A24/A26 (24:02/26:01)	B61/B56 (40:02/56:01)	DR4/DR14 (04:05/14:54)	CD4 (+) / CD8 (-)	A24:02
14	TLHD14	PBMCs	A1/A24 (01:01/24:02)	B37/B51 (37:01/51:01)	DR9/DR10 (09:01/10:01)	CD4 (+) / CD8 (-)	A24:02
15	TLHD15	PBMCs	A2/A24 (02:01/24:02)	B48/B54 (48:01/54:01)	DR4/- (04:05/-)	CD4, nt / CD8, nt	A24:02
16	TLHD16	PBMCs	A2/A31 (02:01/31:01)	B62/B61 (15:01/40:02)	DR9/- (09:01/-)	CD4 (+) / CD8 (+)	

[0043]

[Table 1-2]

17	TLHD17	PBMCs	A24/A31 (24:02/31:01)	B54/B55 (54:01/55:02)	DR4/DR9 (04:05/09:01)	CD4 (+) / CD8 (-)	A24:02
18	TLHD18	PBMCs	A2/A11 (02:01/11:01)	B62/B52 (15:01/52:01)	DR14/DR15 (14:54/15:02)	CD4 (-) / CD8 (-)	B52:01 DRB1 15:02
19	TLHD19	PBMCs	A2/A33 (02:01/33:03)	B35/B44 (35:01/44:03)	DR13/DR15 (13:02/15:02)	CD4 (-) / CD8 (+)	DRB1 15:02
20	TLHD20	PBMCs	A2/A24 (02:01/24:02)	B44/B51 (44:03/51:01)	DR9/DR13 (09:01/13:02)	CD4 (+) / CD8 (+)	A24:02
21	TLHD21	PBMCs	A24/A24 (24:02/24:20)	B39/B52 (39:01/52:01)	DR9/DR15 (09:01/15:02)	CD4 (-) / CD8 (-)	A24:02/B52:01 DRB1 15:02
22	TLHD22	PBMCs	A24/A26 (24:02/26:02)	B39/B61 (39:01/40:06)	DR8/DR14 (08:03/14:06)	CD4 (-) / CD8 (+)	A24:02

* T cell response to 454E2: Cytokine concentration when each T cell (CD4 positive or CD8 positive) is cultured without retinal pigment epithelial cell is taken as control, concentration higher than that is (+) and concentration the same as or lower than that is (-). nt shows not measured.

[0044]

Table 2

No.	RPE and control cells	Origin	HLA-A	HLA-B	HLA-DR	Reference
1	836B1 iPS-RPE cells	iPS cells (skin fibroblasts from HD)	A2/- (02:01/-)	B27/B50 (27:03/50:01)	DR1/13 (01:01/13:03)	
2	101G26 iPS-RPE cells	iPS cells (skin fibroblasts from RP)	A11/A24 (11:01/24:02)	B60/B54 (40:01/54:01)	DR4/DR8 (04:05/08:03)	
3	454E2 iPS-RPE cells	iPS cells (dental pulp cells from HD)	A24/- (24:02/-)	B52/- (52:01/-)	DR15/- (15:02/-)	HLA homozygous donor
4	453F2 iPS-RPE cells	iPS cells (dental pulp cells from HD)	A11/- (11:01/-)	B62/- (15:01/-)	DR4/- (04:06/-)	HLA homozygous donor
5	TLHD1 iPS-RPE cells	iPS cells (skin fibroblasts from HD)	A11/- (11:01/-)	B62/B67 (15:01/67:01)	DR9/DR16 (09:01/16:02)	Autologous TLHD1
6	ES-RPE cells	ES cells	A2/A24 (02:06/24:02)	B35/B39 (35:01/39:01)	DR9/DR14 (09:01/14:05)	
7	Primary RPE cells	Fetal ocular cells	A3/A24 (03:01/24:02)	B35/B60 (35:01/40:01)	DR1/- (01:01/-)	
8	RPE cell lines (ARPE19)	Adult ocular cells	A2/A3 (02:01/03:01)	B7/B65 (07:02/14:02)	DR13/DR15 (13:02/15:01)	
9	Cornea endothelial cells	Primary human CE cells	A24/A68 (24:02/68:01)	B27/B51 (27:03/51:01)	DR7/DR11 (07:01/11:01)	
10	Fibroblast cells	Skin tissues (HD)	A24/A26 (24:02/26:02)	B61/B52 (40:02/52:01)	DR9/DR15 (09:01/15:02)	

5 [0045]

(Preparation Example 3) Preparation of monkey iPS cell-derived RPE cells

iPS cells were established from the fibroblast derived from the skin of Macaca fascicularis according to the method described in Nature (2011) 474: 225-229 (GLIS1use), and monkey iPS cell-derived RPE cells were prepared according to the differentiation induction method described in J Cell Sci (2009)122: 3169-3179 from each of normal monkeys (A, B) and a disease model monkey (C) prepared by forming a retinal

photocoagulation spot in one eye by retinal photocoagulation procedure.

[0046]

(Preparation Example 4) Preparation of monkey T cell suspension

5 CD4 positive T cells and CD8 positive T cells were separated from the peripheral blood of the disease model monkey (C) by using a MACS magnetic cell sorter, and suspended in 100U/ml rhIL-2-containing RPMI culture medium (RPMI 1640 445 ml, FBS 50 ml, penicillin-streptomycin 5 ml) to prepare each T cell
10 suspension.

[0047]

(Reference 2) MHC typing

MHC typing of the cells used in Examples 9, 10 was performed by a conventional method. The disease model monkey
15 (C) had the same alleles at 3 gene loci relative to iPS cell-derived RPE cells (homozygous at 3 gene loci from among Mafa-A, -B, -DRA, -DRB, -DQA, -DQB, -DPA, -DPB gene loci) of normal monkey A. The iPS cell-derived RPE cells of the normal monkey B were different in all genotypes of MHC from the disease model
20 monkey (C).

[0048]

(Example 1)

In a 96 well plate, an anti-CD3 antibody (1 µg/ml) was added to CD4 positive T cell suspensions (TLHD-1 and TLHD-2)
25 obtained in Preparation Example 2 and the suspensions were dispensed to the well at a concentration of 5×10^5 cells/well. 454E2-iPS-derived RPE cells obtained in Preparation Example 1 were added to each well at 3 concentrations of 1×10^3 /well, 5×10^3 /well, 1×10^4 /well, and cultured, wherein the suspension
30 without addition was used as a control. After culture for 48 hr, the culture supernatant was recovered, and the concentration of IFN-γ in the culture supernatant was measured by ELISA. The results are shown in Fig. 1.

[0049]

35 As a result, both TLHD-1 and TLHD-2 showed an increased

production of IFN- γ at all 3 kinds of concentrations as compared to the control. Therefore, transplantation of 454E2-iPS-derived RPE cells to test subjects TLHD-1 and TLHD-2 can be judged to have a high possibility of causing a rejection response.

HLA antigens of TLHD-1 and TLHD-2 showed different haplotype from that of HLA-DR antigen (Class-II) of 454E2-iPS-derived RPE cells. While HLA-A antigen and HLA-B antigen (Class-I) had the same alleles as HLA-A of TLHD-2, other haplotypes were completely different, and all were different in TLHD-1 (Table 3).

[0050]

Table 3

CD4+ cell	iPS-RPE	Class II match	Class I match	IFN- γ
TLHD-1	454E2	mismatch	mismatch	up
TLHD-2	454E2	mismatch	A allele match	up

[0051]

(Example 2)

By a method similar to that in Example 1 except that CD4 positive T cell suspension (test subject names: TLHD-2, TLHD-3, TLHD-4, TLHD-6, TLHD-7, TLHD-8, TLHD-9) obtained in Preparation Example 2 was used as T cell suspension, and 454E2-iPS-derived RPE cells obtained in Preparation Example 1 were used at a concentration of 1×10^4 /well, the production amount of IFN- γ was measured. In addition, using TLHD-1-derived cells instead of T cell suspension and iPS cell-derived RPE cells and by a method similar to that in Example 1, the production amount of IFN- γ was measured. The results are shown in Fig. 2.

[0052]

As a result, the production of IFN- γ was suppressed as compared to control in a combination of T cell suspension of TLHD-1 and iPS cell-derived RPE cells of TLHD-1, and a combination of T cell suspension of TLHD-6 and 454E2-iPS-derived RPE cells.

Therefore, autologous transplantation, or transplantation of 454E2-iPS-derived RPE cells to the test subject TLHD-6 can be judged to have a low possibility of causing a rejection response. That is, the 454E2-iPS-derived RPE cells can be used
5 as a therapeutic agent for an ophthalmic disease of the test subject TLHD-6 or a patient having the same HLA haplotype as test subject TLHD-6, and the same alleles as test subject TLHD-6.

[0053]

10 On the other hand, in the combinations other than of the above, the production amount of IFN- γ increased as compared to the control. Therefore, transplantation of 454E2-iPS cell-derived RPE cells to patients other than a patient with an ophthalmic disease and having the same HLA haplotype as TLHD-6
15 and the same alleles as TLHD-6 can be judged to have a high possibility of causing a rejection response. In this case, either 454E2-iPS cell-derived RPE cells are not utilized for the transplantation or transplantation with combined use of an immunosuppressant needs to be considered.

20 In TLHD-6 that showed suppressed cytokine production, alleles were the same at HLA-DR and HLA-B of 454E2-iPS cell-derived RPE cells. On the other hand, in TLHD-7 that showed high cytokine production, alleles were the same at HLA-A but different at HLA-DR. In TLHD-8, alleles were different from
25 454E2-iPS cell-derived RPE cells. Other cells that showed high cytokine production were different in haplotype from 454E2-iPS cell-derived RPE cells (Table 4).

[0054]

[Table 4]

CD4+ cell	iPS-RPE	Class II match	Class I match	IFN- γ
TLHD-1	TLHD-1	All match	All match	down
TLHD-6	454E2	DR allele match	B allele match	down
TLHD-7	454E2	DR haplotype match	A allele match	up
TLHD-8	454E2	DR haplotype match	mismatch	up
TLHD-2	454E2	mismatch	A allele match	up
TLHD-3	454E2	mismatch	mismatch	up
TLHD-4	454E2	mismatch	mismatch	up
TLHD-9	454E2	mismatch	A allele match	up

[0055]

(Example 3)

5 By a method similar to that in Example 1 except that CD4 positive T cell suspension (TLHD-1) obtained in Preparation Example 2 was used as T cell suspension, and Nos. 1 - 3, 6 - 10 shown in Table 2 and mouse fibroblast were used at a concentration of 1×10^4 /well instead of 454E2-iPS-derived RPE cells obtained in Preparation Example 1, the production amount of IFN- γ was measured. The results are shown in Fig. 3.

[0056]

As a result, in all cells other than human fibroblast, the production amount of IFN- γ increased. Therefore, 15 transplantation of 454E2-iPS cell-derived RPE cells to patients other than a patient with an ophthalmic disease and having the same HLA haplotype as the above-mentioned human fibroblast and the same alleles as the above-mentioned human fibroblast can be judged to have a high possibility of causing a rejection response. In this case, either 454E2-iPS cell-derived RPE 20 cells are not utilized for the transplantation or transplantation with combined use of an immunosuppressant can be considered.

Relative to HLA-DR antigen of ES-derived RPE cells and 25 human fibroblast, TLHD-1 had the same alleles, and others were

different in haplotype (Table 5).

[0057]

[Table 5]

Lane	CD4+ cell	Cell	Class II match	IFN- γ
2	TLHD-1	836B1 iPS-RPE	mismatch	up
3	TLHD-1	101G26 iPS-RPE	mismatch	up
4	TLHD-1	454E2 iPS-RPE	mismatch	up
5	TLHD-1	ES-RPE	DR allele match	up
6	TLHD-1	fetal RPE	mismatch	up
7	TLHD-1	ARPE-19	mismatch	up
8	TLHD-1	HCE	mismatch	up
9	TLHD-1	human fibroblast	DR allele match	same
10	TLHD-1	mouse fibroblast	mismatch	up

5 [0058]

(Example 4)

A similar experiment was performed except that CD4 positive T cell suspension (test subject names: TLHD-1, TLHD-8, TLHD-14, TLHD-21) obtained in Preparation Example 2 was used as
10 T cell suspension, and 453F2-iPS-derived RPE cells shown in Table 2 were used instead of 454E2-iPS-derived RPE cells obtained in Preparation Example 1. The results are shown in Fig. 4.

Alternatively, a similar experiment was performed except
15 that CD8 positive T cell suspension (test subject names: TLHD-1, TLHD-8, TLHD-14, TLHD-22) obtained in Preparation Example 2 was used as T cell suspension in Example 1, and 453F2-iPS-derived RPE cells shown in Table 2 were used instead of 454E2-iPS-derived RPE cells obtained in Preparation Example 1. The
20 results are shown in Fig. 5.

[0059]

As a result, the production of Granzyme B was suppressed as compared to control in a combination of CD8 positive T cell suspension of TLHD-1 and 453F2-iPS-derived RPE cells.
25 Therefore, transplantation of 453F2-iPS-derived RPE cells to

the test subject TLHD-1 can be judged to have a low possibility of causing a rejection response. That is, the 453F2-iPS-derived RPE cells can be used as a therapeutic agent for an ophthalmic disease of test subject TLHD-1 or a patient having the same HLA haplotype as the test subject TLHD-1, and the same alleles as the test subject TLHD-1.

[0060]

(Example 5)

In a 96 well plate, an anti-CD3 antibody was added at 1 μ g/ml to CD4 positive T cell suspension (TLHD-1) obtained in Preparation Example 2 and dispensed to the well at a concentration of 5×10^5 cells/well. 454E2-iPS-derived RPE cells homozygous at 3 gene loci of HLA obtained in Preparation Example 1 were added to each well at a concentration of 1×10^4 /well, and cultured, wherein the suspension without addition was used as a control. After culture for 48 hr, the cells were recovered, and the production of IL-2, IFN- γ , IL-17, TNF- α , IL-22 in CD4 positive T cells was respectively measured by FACS. The results are shown in Fig. 6.

[0061]

As a result, it was found that IFN- γ , IL-17 and TNF- α can be utilized as an index of cytokine production in anti-CD4 positive T cells.

[0062]

In addition, a similar experiment was further performed using the CD4 positive T cell suspensions (TLHD-1 and TLHD-10) obtained in Preparation Example 2, and various cytokine production amounts were compared (Fig. 7a and b).

As a result, among many cytokines, the expression level of IFN- γ alone decreased when 454E2-iPS cell-derived RPE cells (homozygous at 3 gene loci of HLA-A, HLA-B, HLA-DR) were used for T cells (alleles of all genotypes of 3 gene loci of HLA-A, HLA-B, HLA-DR are same) of the test subject name TLHD-10. Thus, it was found that IFN- γ is suitable as an index of whether or not a rejection response occurs.

[0063]

(Example 6)

In a 96 well plate, an anti-CD8 antibody was added at 1
µg/ml to CD8 positive T cell suspensions (TLHD-1) obtained in
5 Preparation Example 2 and dispensed to the well at a
concentration of 1×10^5 cells/well. 454E2-iPS-derived RPE
cells obtained in Preparation Example 1 were added to each well
at a concentrations of 1×10^4 /well, and cultured, wherein the
suspension without addition was used as a control. After
10 culture for 48 hr, the cells were recovered, and the production
of Granzyme B, Perforin, TNF-α in the CD8 positive T cells was
each measured by ELISA. The ratio of double positive cells is
shown in Fig. 8.

As a result, it was suggested that a cytotoxic substance
15 produced by CD8 positive T cells can be used for screening for
a rejection response.

[0064]

(Example 7)

A method similar to that in Example 1 was performed
20 except that suspensions of CD8 positive T cells (test subject
names: TLHD-1, TLHD-3) were used instead of CD4 positive T
cells and production of Granzyme B was measured instead of IFN-
γ. The results are shown in Fig. 9.

[0065]

25 As a result, both TLHD-1 and TLHD-3 showed an increased
production of Granzyme B at all 2 kinds of concentrations as
compared to the control. Therefore, transplantation of 454E2-
iPS-derived RPE cells to test subjects TLHD-1 and TLHD-3 can be
judged to have a high possibility of causing a rejection
30 response.

HLA antigens of TLHD-1 and TLHD-3 showed different
haplotype from that of both HLA-A antigen and HLA-B antigen of
454E2-iPS-derived RPE cells (Table 6).

[0066]

35 [Table 6]

CD8+Tcell	iPS-RPE	Class I match	Class II match	Granzyme B
TLHD-1	454E2	mismatch	mismatch	up
TLHD-3	454E2	mismatch	mismatch	up

[0067]

(Example 8)

A method similar to that in Example 1 was performed except that suspensions of CD8 positive T cells (test subject names: TLHD-2, 4, 5, 8, 10, 12, 13, 14, 16, 17, 18, 19, 20) were used instead of CD4 positive T cells and production of Granzyme B was measured instead of IFN- γ . The results are shown in Fig. 10.

10 [0068]

As a result, the production of Granzyme B was suppressed as compared to control in a combination of T cell suspensions of TLHD-10, TLHD-18, TLHD-2, TLHD-14, TLHD-12, TLHD-13, TLHD-17 and 454E2-iPS-derived RPE cells. Therefore, transplantation of 454E2-iPS-derived RPE cells to the test subjects TLHD-10, TLHD-18 can be judged to have a low possibility of causing a rejection response. While TLHD-2, TLHD-14, TLHD-12, TLHD-13, TLHD-17 matched only with the alleles of HLA-A, the possibility of not causing a rejection response needs to be considered.

20 [0069]

On the other hand, in the combinations other than of the above, the production amount of Granzyme B increased as compared to the control. Therefore, transplantation of 454E2-iPS cell-derived RPE cells to the corresponding test subjects can be judged to have a high possibility of causing a rejection response. In this case, either 454E2-iPS cell-derived RPE cells are not utilized for the transplantation or transplantation with combined use of an immunosuppressant can be considered.

30 [0070]

In TLHD-10 and TLHD-18 that showed suppressed production of Granzyme B, both the haplotypes and alleles matched in HLA-A

or HLA-B, and HLA-DR. On the other hand, in TLHD-2, TLHD-14, TLHD-12, TLHD-13, haplotype and alleles were the same at HLA-A or HLA-B of 454E2-iPS cell-derived RPE cells. On the other hand, also in TLHD-5 and TLHD-20 that showed increased
5 production of Granzyme B, haplotype and alleles were the same at HLA-A or HLA-B of 454E2-iPS cell-derived RPE cells, and in TLHD-8, only HLA-DR haplotype was the same (Table 7).

[0071]

Thus, even when allele and haplotype partially match,
10 production of Granzyme B may increase. However, when at least one of the alleles of HLA-A, HLA-B or and HLA-DR is the same, it can be judged that the possibility of causing a rejection response is low. Furthermore, when at least any two of the alleles of HLA-A, HLA-B or HLA-DR are the same, it can be
15 judged that the possibility of causing a rejection response is lower, and when all alleles of HLA-A, HLA-B and HLA-DR are the same, it can be judged that the cells are suitable for transplantation. On the contrary case, either the cells are not utilized for the transplantation or the case can be used as
20 an index for consideration of transplantation with combined use of an immunosuppressant. Other cells that showed high granzyme B production were different in haplotype from 454E2-iPS cell-derived RPE cells.

[0072]

25 [Table 7]

CD8+Tcell	iPS-RPE	Class I match	Class II match	Granzyme B
TLHD-10	454E2	A allele match, B allele match	DR allele match	down
TLHD-18	454E2	B allele match	DR allele match	down
TLHD-2	454E2	A allele match	mismatch	down
TLHD-14	454E2	A allele match	mismatch	down
TLHD-12	454E2	A allele match	mismatch	down
TLHD-13	454E2	A allele match	mismatch	down
TLHD-17	454E2	A allele match	mismatch	down
TLHD-5	454E2	A allele match	mismatch	up
TLHD-20	454E2	A allele match	mismatch	up
TLHD-8	454E2	mismatch	DR haplotype match	up
TLHD-4	454E2	mismatch	mismatch	up
TLHD-19	454E2	mismatch	DR allele match	up
TLHD-16	454E2	mismatch	mismatch	up

[0073]

(Example 9)

(prediction test)

5 In a 96 well plate, an anti-CD3 antibody is added at 1 µg/ml to CD4 positive T cell suspension (monkey disease model C) obtained in Preparation Example 4 and the suspension was dispensed to the well at a concentration of 5×10^5 cells/well. Normal monkey B-iPS-derived RPE cells or disease model monkey
10 C-iPS-derived RPE cells obtained in Preparation Example 3 are added to each well at 1×10^4 /well, and cultured, wherein the suspension without addition was used as a control. After culture for 48 hr, the culture supernatant is recovered, and the concentration of IFN-γ in the culture supernatant is
15 measured by ELISA.

A method similar to the aforementioned method is performed except that a suspension of CD8 positive T cells (monkey disease model C) is used instead of CD4 positive T cells and production of Granzyme B is measured instead of IFN-γ.

20 In the case of normal monkey B-iPS-derived RPE cells, the production amounts of both IFN-γ and Granzyme B increase as

compared to the control, whereas in the case of disease model monkey C-iPS-derived RPE cells, the production amounts of both IFN- γ and Granzyme B are suppressed as compared to the control. [0074]

5 (transplantation test)

Normal monkey B-iPS-derived RPE cells or disease model monkey C-iPS-derived RPE cells are transplanted to one eye of disease model monkey C, according to the method described in Invest Ophthalmol Vis Sci.1995 Feb; 36(2):381-90. On day 28
10 after transplantation, a section of fundus is imaged like a tissue section by using fundus photograph and OCT (Optical coherence tomograph), and the condition of the retina is confirmed.

Allogeneic transplantation of normal monkey B-iPS-derived
15 RPE cells shows clear rejection responses such as fibrotic change of graft periphery, leakage of fluorescence by fluorescein fundus angiography, and high-intensity lesion under retina in OCT. Allogeneic transplantation of disease model monkey C-iPS-derived RPE cells does not show such clear
20 rejection response, leakage of fluorescence by fluorescein fundus angiography is absent, graft is engrafted, and disorders such as thinning of sensory retina and the like do not occur. [0075]

(Example 10)

25 (prediction test)

A method similar to that in Example 8 is performed except that normal monkey A-iPS-derived RPE cells (homozygous at 3 gene loci of MHC) obtained in Preparation Example 3 or disease model monkey C-iPS-derived RPE cells are used at a
30 concentration of 1×10^4 /well, and the production amount of IFN- γ is measured.

A method similar to the aforementioned method is performed except that a suspension of CD8 positive T cells (monkey disease model C) is used instead of CD4 positive T
35 cells and production of Granzyme B is measured instead of IFN- γ .

In both normal monkey A-iPS-derived RPE cells and disease model monkey C-iPS-derived RPE cells, the production amounts of both IFN- γ and Granzyme B are suppressed as compared to the control.

5 [0076]

(transplantation test)

A method similar to the transplantation test in Example 8 is performed except that normal monkey A-iPS-derived RPE cells (homozygous at 3 gene loci) or disease model monkey C-iPS-
10 derived RPE cells are used as iPS-derived RPE cells, iPS-derived RPE cells are transplanted to one eye of disease model monkey C. On day 28 after transplantation, a section of fundus is imaged like a tissue section by using fundus photograph and OCT (Optical coherence tomograph), and the condition of the
15 retina is confirmed.

Allogeneic transplantation of normal monkey A-iPS-derived RPE cells (homozygous at 3 gene loci) shows no clear rejection response, like autologous transplantation of disease model monkey C-iPS-derived RPE cells, leakage of fluorescence by
20 fluorescein fundus angiography is absent, graft is engrafted, and disorders such as thinning of sensory retina and the like do not occur.

[Industrial Applicability]

[0077]

25 According to the present invention, however, when the genotype of 3 gene loci of HLA-A, HLA-B and HLA-DR on the donor side is homozygous in RPE cell transplantation, rejection can be suppressed by merely matching one of the HLA loci of the recipient. Since immunologically incompatible cells for
30 transplantation can be distinguished and selected by previously collecting blood samples, the burden on patients can be reduced by avoiding incompatible cells for transplantation. The cells selected by this method can be used for RPE cell transplantation. In addition, the present invention used to
35 determine whether an immunosuppressant is necessary can

contribute to the selection of a treatment plan. Thus, the present invention contributes to marked improvement of treatment efficiency by allogeneic transplantation, and also affords effects such as reduction of the preparation costs of
5 cells unsuitable for transplantation and the like.

This application is based on patent application No. 2014-032325 filed in Japan (filing date: February 21, 2014), the contents of which are incorporated in full herein.

Claims

1. A therapeutic agent for ophthalmic diseases, comprising a retinal pigment epithelial cell having an HLA-A gene locus, an
5 HLA-B gene locus and an HLA-DR gene locus, wherein each locus has a homozygous genotype, which agent is for a patient with an ophthalmic disease and having a combination wherein at least one of the alleles at each corresponding gene locus is the same as an allele at the gene locus of the retinal pigment
10 epithelial cell.
2. The agent according to claim 1, which suppresses an immunorejection response after transplantation.
- 15 3. The agent according to claim 1 or 2, wherein the retinal pigment epithelial cell is derived from a pluripotent stem cell.
4. The agent according to claim 3, wherein the pluripotent stem cell is an iPS cell.
- 20 5. The agent according to any one of claims 1 to 4, wherein the ophthalmic disease is retina denaturation or a disease associated with retina denaturation.
- 25 6. A method of selecting a therapeutic agent for an ophthalmic disease for a patient with the ophthalmic disease, comprising (i) a step of examining genotypes of HLA-A gene locus, HLA-B gene locus and HLA-DR gene locus of patient-derived T cells, (ii) a step of selecting a retinal pigment epithelial cell
30 having an allele matching the allele at each gene locus clarified in step (i).
7. The method according to claim 6, wherein the retinal pigment epithelial cell in step (ii) has an HLA-A gene locus, an HLA-B
35 gene locus and an HLA-DR gene locus, and a genotype of each

locus is homozygous.

8. A method of selecting a therapeutic agent for ophthalmic diseases, which is for a patient with an ophthalmic disease,
5 comprising

(1) a step of performing contact culture of patient-derived T cells with retinal pigment epithelial cells,
(2) a step of measuring a concentration of inflammatory cytokine secreted in the culture supernatant in step (1), and
10 (3) a step of judging that the retinal pigment epithelial cell can be used as an active ingredient of a therapeutic agent for ophthalmic diseases of a patient, when the inflammatory cytokine concentration in step (2) is of the same level as or not more than a concentration of an inflammatory cytokine in a
15 culture supernatant obtained by culturing the patient-derived T cells alone.

9. The method according to claim 8, wherein the retinal pigment epithelial cell has an HLA-A gene locus, an HLA-B gene locus
20 and an HLA-DR gene locus, and a genotype of each locus is homozygous.

10. The method according to claim 8 or 9, wherein the patient-derived T cells in step (1) are activated using an anti-CD3
25 antibody.

11. The method according to any one of claims 8 to 10, wherein the patient-derived T cells in step (1) are CD4 positive, and the inflammatory cytokine measured in step (2) is IFN- γ .

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12. The method according to any one of claims 8 to 10, wherein the patient-derived T cells in step (1) are CD8 positive, and the inflammatory cytokine measured in step (2) is Granzyme B.

35 13. A test method for predicting a rejection response in an

ophthalmic disease patient to be transplanted with a retinal pigment epithelial cell for transplantation, comprising (A) a step of performing contact culture of patient-derived T cells with said retinal pigment epithelial cells,

- 5 (B) a step of measuring a concentration of inflammatory cytokine secreted in the culture supernatant in step (A), and (C) a step of judging that a rejection response to the retinal pigment epithelial cell occurs, when the inflammatory cytokine concentration in step (B) is significantly higher than a
10 concentration of an inflammatory cytokine in a culture supernatant obtained by culturing the patient-derived T cells alone.

14. The method according to claim 13, wherein the retinal
15 pigment epithelial cell for transplantation has an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, and a genotype of each locus is homozygous.

15. The method according to claim 13 or 14, wherein the
20 patient-derived T cells in step (A) are activated using an anti-CD3 antibody.

16. The method according to any one of claims 13 to 15, wherein the patient-derived T cells in step (A) are CD4 positive, and
25 the inflammatory cytokine measured in step (B) is IFN- γ .

17. The method according to any one of claims 13 to 15, wherein the patient-derived T cells in step (A) are CD8 positive, and the inflammatory cytokine measured in step (B) is Granzyme B.

30

18. A method of treating an ophthalmic disease, comprising forming a sheet of a retinal pigment epithelial cell having an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, wherein each locus has a homozygous genotype, and transplanting
35 the sheet to a patient with an ophthalmic disease and having a

combination wherein at least one of the alleles at each corresponding gene locus is the same as an allele at the gene locus of the retinal pigment epithelial cell.

5 19. The method according to claim 18, wherein the retinal pigment epithelial cell is derived from a pluripotent stem cell.

20. The method according to claim 19, wherein the pluripotent stem cell is an iPS cell.

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21. The method according to any one of claims 18 to 20, wherein the ophthalmic disease is retina denaturation or a disease associated with retina denaturation.

15 22. A retinal pigment epithelial cell having an HLA-A gene locus, an HLA-B gene locus and an HLA-DR gene locus, wherein each locus has a homozygous genotype, which cell is for use for the treatment of an ophthalmic disease of a patient with an ophthalmic disease and having a combination wherein at least
20 one of the alleles at each corresponding gene locus is the same as an allele at the gene locus of the retinal pigment epithelial cell.

23. The retinal pigment epithelial cell according to claim 22,
25 wherein the retinal pigment epithelial cell is derived from a pluripotent stem cell.

24. The retinal pigment epithelial cell according to claim 23, wherein the pluripotent stem cell is an iPS cell.

30

25. The retinal pigment epithelial cell according to any one of claims 22 to 24, wherein the ophthalmic disease is retina denaturation or a disease associated with retina denaturation.

35 26. A retinal pigment epithelial cell having an HLA-A gene

locus, an HLA-B gene locus and an HLA-DR gene locus, wherein each locus has a homozygous genotype, which cell is for use for the production of a therapeutic agent for an ophthalmic disease of a patient with an ophthalmic disease and having a
5 combination wherein at least one of the alleles at each corresponding gene locus is the same as an allele at the gene locus of the retinal pigment epithelial cell.

27. The retinal pigment epithelial cell according to claim 26,
10 wherein the retinal pigment epithelial cell is derived from a pluripotent stem cell.

28. The retinal pigment epithelial cell according to claim 27, wherein the pluripotent stem cell is an iPS cell.

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29. The retinal pigment epithelial cell according to any one of claims 26 to 28, wherein the ophthalmic disease is retina denaturation or a disease associated with retina denaturation.