

DE 10235310 discloses a test in which the activation of basophils is detected using a fluorescent calcium dye. Blood fractionated by density gradient centrifugation is used.

WO 2012/044756, US 2009/0246805, WO 98/32014 and EP 2037269 disclose flow cytometric tests for detecting the activation of basophils.

Against this background, an object of the invention is to provide a method and reagents suitable for said method by means of which the above-mentioned drawbacks of the methods described in the prior art can be overcome, in particular a basophil activation test that can be carried out with a system other than a flow cytometer.

In particular, an object is to provide a system that allows the *simultaneous* processing of a plurality of samples in a high-throughput method. Furthermore, reproducibility and standardizability are to be ensured.

This and further objects are achieved by the subject matter of the present invention, in particular by the subject matter of the attached independent claims, wherein preferred embodiments are given in the dependent claims.

Summary

In a first aspect, the present disclosure refers to an *in vitro* method for diagnosing an allergy, comprising the following steps: a) bringing into contact of basophils obtained from a whole blood sample of a patient with an allergen in an aqueous solution in the presence of whole blood, with a volume content of whole blood in the aqueous solution of at least 20 percent by volume, under conditions that allow activation of the basophils by the allergen, b) enrichment of the basophils from step a) by binding the basophils to (1) a first monoclonal antibody that binds a first cell surface polypeptide characteristic of activated basophils, or (2) a first monoclonal antibody that binds a first basophil cell surface polypeptide independent of activation status of basophils, and c) detection of activated basophils if present in the enriched basophil from step b) by chemiluminescence, wherein (1) if step b) (1) is performed, step c) is carried out by detecting the activated basophils using a second antibody that binds a second cell surface polypeptide characteristic of activated basophils or a second antibody that binds a second basophil cell surface polypeptide independent of activation status of basophils, or (2) if step b) (2) is performed, step c) is carried out by detecting the activated basophils using a second antibody that binds a second cell surface polypeptide characteristic of activated basophils, wherein either the first or second antibody is labeled with a label capable of chemiluminescence or the activated basophils are

detected by a secondary antibody with a label capable of chemiluminescence binding to the first monoclonal antibody or the second antibody.

In another aspect, the present disclosure refers to a kit comprising a vessel for bringing the basophils into contact with the allergen, wherein the vessel is preferably a microtiter plate, optionally an allergen, and a monoclonal antibody against a polypeptide located on the cell surface of basophils for the enrichment of basophils, wherein the monoclonal antibody against the polypeptide on the cell surface of basophils subjects the basophils to immobilization on the vessel, and an antibody against a marker for detection located on the cell surface of basophils, wherein the antibody against the marker for detection located on the surface of basophils comprises a label capable of chemiluminescence, wherein the label capable of chemiluminescence can be an enzyme that catalyzes a chemiluminescence reaction or a molecule that itself produces a chemiluminescence signal under suitable conditions, wherein the enzyme in the former case is contained in the kit in combination with a substrate solution capable of chemiluminescence, and wherein the polypeptide located on the cell surface of basophils and/or the marker located on the cell surface of basophils is characteristic of activated basophils, optionally an agent for stopping activation of the basophils, and optionally an agent for lysing and/or removing erythrocytes.

In yet another aspect, the present disclosure refers to the use of a monoclonal antibody against a polypeptide located on the cell surface of basophils for the enrichment of basophils, wherein the monoclonal antibody against the polypeptide on the cell surface of basophils subjects the basophils to immobilization on a vessel, and an antibody against a marker for detection located on the cell surface of basophils a) in the manufacture of a medicament for combination therapy for treating an allergy to an allergen, or b) diagnosing an allergy, or c) for screening candidate active ingredients for the treatment of an allergy to an allergen; wherein the antibody against the marker for detection located on the surface of basophils comprises a label capable of chemiluminescence, wherein the label capable of chemiluminescence can be an enzyme that catalyzes a chemiluminescence reaction or a molecule that itself produces a chemiluminescence signal under suitable conditions, wherein the enzyme in the former case is used in combination with a substrate solution capable of chemiluminescence, and wherein the polypeptide located on the cell surface of basophils and/or the marker located on the cell surface of basophils is characteristic of activated basophils.

Detailed Description

In a first aspect, the problem of the invention is solved by means of a method for diagnosing an allergy, preferably a method suitable for high throughput, comprising the following steps:

- a) bringing into contact of basophils from a whole blood sample of a patient with an allergen in an aqueous solution in the presence of whole blood, with a volume content of whole blood in the aqueous solution of at least 20 percent by volume, under conditions that allow activation of the basophils by the allergen,
- b) enrichment of the basophils from step a) and
- c) detection of activated basophils,

wherein the detection in step c) is carried out in that a marker characteristic of activated basophils is detected by means of chemiluminescence.

Alternatively, the problem is solved in a first aspect by means of a method for diagnosing an allergy, comprising the following steps:

Claims

1. An *in vitro* method for diagnosing an allergy, comprising the following steps:
 - a) bringing into contact of basophils obtained from a whole blood sample of a patient with an allergen in an aqueous solution in the presence of whole blood, with a volume content of whole blood in the aqueous solution of at least 20 percent by volume, under conditions that allow activation of the basophils by the allergen,
 - b) enrichment of the basophils from step a) by binding the basophils to
 - (1) a first monoclonal antibody that binds a first cell surface polypeptide characteristic of activated basophils, or
 - (2) a first monoclonal antibody that binds a first basophil cell surface polypeptide independent of activation status of basophils, and
 - c) detection of activated basophils if present in the enriched basophil from step b) by chemiluminescence, wherein
 - (1) if step b) (1) is performed, step c) is carried out by detecting the activated basophils using a second antibody that binds a second cell surface polypeptide characteristic of activated basophils or a second antibody that binds a second basophil cell surface polypeptide independent of activation status of basophils, or
 - (2) if step b) (2) is performed, step c) is carried out by detecting the activated basophils using a second antibody that binds a second cell surface polypeptide characteristic of activated basophils,
- wherein either the first or second antibody is labeled with a label capable of chemiluminescence or the activated basophils are detected by a secondary antibody with a label capable of chemiluminescence binding to the first monoclonal antibody or the second antibody.

2. The method as claimed in claim 1, wherein the whole blood is unprocessed whole blood.
3. The method as claimed in one of claims 1 and 2, wherein step a) is carried out in an aqueous solution with a whole blood content of at least 30, preferably 35, more preferably 40, more preferably 45 percent by volume.
4. The method of one of claims 1 through 3, comprising the step of stopping the activation of the basophils, preferably before step b).
5. The method as claimed in one of claims 1 through 4, wherein in step b), the basophils are immobilized
6. The method as claimed in claim 5, wherein the basophils are immobilized on a bead, preferably a magnetic bead.
7. The method as claimed in one of claims 5 through 6, wherein the basophils are washed after immobilization.
8. The method as claimed in one of claims 1 through 7, wherein in step b), erythrocytes are lysed and removed.
9. The method as claimed in claim 8, wherein the erythrocytes are removed before immobilization of the basophils by centrifugation.
10. The method as claimed in claim 1 through 9, wherein steps a), b) and c) are carried out in a well of a microtiter plate.
11. A kit comprising

a vessel for bringing the basophils into contact with the allergen, wherein the vessel is preferably a microtiter plate,

optionally an allergen, and

a monoclonal antibody against a polypeptide located on the cell surface of basophils for the enrichment of basophils, wherein the monoclonal antibody against the polypep-

tion on the cell surface of basophils subjects the basophils to immobilization on the vessel, and an antibody against a marker for detection located on the cell surface of basophils,

wherein the antibody against the marker for detection located on the surface of basophils comprises a label capable of chemiluminescence,

wherein the label capable of chemiluminescence can be an enzyme that catalyzes a chemiluminescence reaction or a molecule that itself produces a chemiluminescence signal under suitable conditions, wherein the enzyme in the former case is contained in the kit in combination with a substrate solution capable of chemiluminescence,

and wherein the polypeptide located on the cell surface of basophils and/or the marker located on the cell surface of basophils is characteristic of activated basophils,

optionally an agent for stopping activation of the basophils, and

optionally an agent for lysing and/or removing erythrocytes.

12. Use of a monoclonal antibody against a polypeptide located on the cell surface of basophils for the enrichment of basophils, wherein the monoclonal antibody against the polypeptide on the cell surface of basophils subjects the basophils to immobilization on a vessel, and an antibody against a marker for detection located on the cell surface of basophils

- a) in the manufacture of a medicament for combination therapy for treating an allergy to an allergen, or

- b) for diagnosing an allergy, or

- c) for screening candidate active ingredients for the treatment of an allergy to an allergen;

wherein the antibody against the marker for detection located on the surface of basophils comprises a label capable of chemiluminescence,

wherein the label capable of chemiluminescence can be an enzyme that catalyzes a chemiluminescence reaction or a molecule that itself produces a chemiluminescence signal under suitable conditions, wherein the enzyme in the former case is used in combination with a substrate solution capable of chemiluminescence,

and wherein the polypeptide located on the cell surface of basophils and/or the marker located on the cell surface of basophils is characteristic of activated basophils.