A method of treating atherosclerosis comprises removing AGE-modified cells from a patient. The AGE-modified cells include erythrocytes, intima cells, endothelial cells, smooth muscle cells, macrophages, and foam cells. A variety of techniques, such as ultrasound and binding with an anti-AGE antibody, may be used to identify and remove the AGE-modified cells.
SELECTIVE REMOVAL OF AGE-MODIFIED CELLS FOR TREATMENT OF ATHEROSCLEROSIS

CROSS REFERENCE TO RELATED APPLICATION

[01] This application claims priority to provisional application no. 61/386,932 entitled "SELECTIVE REMOVAL OF AGE-MODIFIED CELLS FOR TREATMENT OF ATHEROSCLEROSIS" filed 27-September-2010, attorney docket no. SIW01-002-PRO, the entire contents of which are hereby incorporated by reference, except where inconsistent with the present application.

BACKGROUND


[03] AGE-modified erythrocytes have less flexibility than non-modified erythrocytes, and have been implicated in the pathogenesis of atherosclerosis, while the absence of AGE-modified erythrocytes has been correlated with reduced atherosclerosis. Jandeleit-Dahm K, et al./, "The AGE/RAGE Axis in Diabetes-Accelerated Atherosclerosis," Clinical and Experimental Pharmacology and Physiology, Vol. 35, 329-334 (2008) at 330. Localization of AGEs in atherosclerotic lesions of the aorta in non-diabetic patients has been reported in intima cells,

SUMMARY

[04] In a first aspect, the present invention is a method of treating atherosclerosis comprising removing AGE-modified cells from a patient.

[05] In a second aspect, the present invention is a method of removing AGE-modified erythrocytes from blood, comprising damaging or destroying an AGE-modified erythrocyte with ultrasound.

[06] In a third aspect, the present invention is a method of removing AGE-modified cells, comprising binding the AGE-modified cells with an anti-AGE monoclonal antibody.

[07] In a fourth aspect, the present invention is a method of removing AGE-modified cells from atherosclerotic lesions, comprising binding the AGE-modified cells with an anti-AGE monoclonal antibody.

[08] Definitions

[09] The following definitions are included to provide a clear and consistent understanding of the specification and claims.

[10] The term "advanced glycation end-products" refers to the aggregate of glycated proteins on the cell membrane that are formed as the result of the reaction of sugars with protein side chains, and are also referred to as AGE-modified proteins and AGE-modified cells.
DETAILED DESCRIPTION

[11] The present invention makes use of the discovery that enhanced clearance of AGE-modified cells, such as erythrocytes, is beneficial in reducing cardiovascular disease, especially when present as a complication of diabetes, or the pre-diabetic condition referred to as "Syndrome-X". Elevated blood glucose concentrations lead to modifications of protein side chains in cells, including circulating erythrocytes and other cell types. Non-enzymatic glycation of membrane proteins results in the formation of AGE-modified cells, which cause reduced cell deformability that is associated with the formation of atherosclerotic lesions.

[12] The technique for removing AGE-modified erythrocytes from a patient is selected for its ability to detect and selectively remove or destroy AGE-modified cells while avoiding removal or destruction of cells that are not AGE-modified. For example, AGE-modified erythrocytes may be detected due to their increased stiffness and reduced deformability by ultrasound. In an example, ultrasound treatment may be applied at driving frequencies ranging from 1.0 Mhz to 5.0 Mhz, preferably from 3.0 Mhz to 4.0 Mhz. Time of exposure may range from three to sixty minutes daily for up to 20 days.

[13] Additionally, anti-AGE monoclonal antibodies may be used for their ability to selectively bind AGE-modified cells. Anti-AGE monoclonal antibodies bind to AGE-modified cells, such as AGE-modified erythrocytes, to selectively remove the AGE-modified cells from a patient. The blood from the patient may be passed through extracorporeal circulation and AGE-modified erythrocytes are then bound by anti-AGE monoclonal antibodies attached to a solid substrate via their Fc region.

[14] Further, anti-AGE monoclonal antibodies covalently conjugated to a fluorescent marker may be used to label AGE-modified erythrocytes that are then removed from the patient's blood via cell sorting. An anti-AGE monoclonal antibody is injected into the patient to label AGE-modified erythrocytes and, subsequently, the patient's blood is connected to a cell sorter via extracorporeal circulation tubing.
system. AGE-modified erythrocytes bound to a fluorescent anti-AGE monoclonal antibody are sorted from normal erythrocytes and other blood cell types.

Anti-AGE monoclonal antibodies can be conjugated to an agent that causes the destruction of AGE-modified cells. Such agent can be, but is not limited to a toxin, a cytotoxic agent, magnetic nanoparticles, and magnetic spin-vortex discs.

Moreover, AGE-modified cell types localized in atherosclerotic lesions of the aorta in non-diabetic patients, such as intima cells, endothelial cells, smooth muscle cells, macrophages, and foam cells, may be selectively removed by using anti-AGE monoclonal antibodies conjugated to an agent that causes the destruction of AGE-modified cells. Such agent can be, but is not limited to a toxin, a cytotoxic agent, magnetic nanoparticles, and magnetic spin-vortex discs.

A toxin, such as pore-forming toxins (PFT) (Aroian R. et al., "Pore-Forming Toxins and Cellular Non-Immune Defenses (CNIDs)," Current Opinion in Microbiology, 10:57-61 (2007)), conjugated to an anti-AGE monoclonal antibody may be injected into a patient to selectively target and remove AGE-modified cells. The anti-AGE monoclonal antibody recognizes and binds to AGE-modified erythrocytes or AGE-modified cells present in atherosclerotic lesions. Then, the toxin causes pore formation at the cell surface and subsequent cell removal through osmotic lysis (Id. at p.58).

Magnetic nanoparticles conjugated to anti-AGE monoclonal antibodies may be injected into a patient to target and remove AGE-modified erythrocytes or AGE-modified cells present in atherosclerotic lesions. The magnetic nanoparticles can be heated by applying a magnetic field in order to selectively remove the AGE-modified erythrocytes or AGE-modified cells present in atherosclerotic lesions.

As an alternative, magnetic spin-vortex discs, which are magnetized only when a magnetic field is applied to avoid self-aggregation that can block blood vessels, begin to spin when a magnetic field is applied, causing membrane disruption of target cells. Magnetic spin-vortex discs, conjugated to anti-AGE
monoclonal antibodies specifically target AGE-modified cell types, without removing other cells.

[20] EXAMPLES

[21] Example 1 (Prophetic) Ultrasound Removal of AGE-Modified Erythrocytes in ZDF (Zucker Diabetic Fatty) Rats

In this example ZDF rats, a type II diabetic rat model demonstrating obesity, insulin resistance, hyperinsulinemia, hyperglycemia, hypertriglyceridemia, hypercholesterolemia, nephropathy, impaired wound healing, mild hypertension, and neuropathy, are exposed to ultrasound to determine (1) the background level of glycated hemoglobin A1c in this strain; (2) whether exposure to ultrasound at clinical imaging levels, is tolerable by assessing clinical observation on the animals; (3) whether there is an effect on the level of glycated hemoglobin A1c due to exposure to ultrasound. The level of glycated hemoglobin A1c is used as a marker for removal of AGE-modified erythrocytes.

Ten ZDF rats, approximately eight weeks old at receipt, supplied by Charles River Laboratories (Wilmington, MA) are randomly assigned in two groups, labeled I and II. The animals are weighed prior to ultrasound exposure. The rats are shaved dorsally and ultrasound gel is applied by pressing and rubbing the applicator across the dorsal aspect of the rat, from thorax from tail. While one technician holds the animal, another uses the applicator. The ultrasound machine is set at 3.3 Mhz and the applicator is pressed against the dorsal aspect of the animal and moved slowly from thorax to tail for the appropriate time of exposure. Rats in group I are exposed to five minutes of ultrasound at 3.3 Mhz/day for ten days and rats in group II are exposed to ten minutes of ultrasound at 3.3 Mhz/day for ten days. Following exposure the animal is wiped off to remove ultrasound gel and placed back in its cage. The animals remain under observation for four hours within four hours from exposure for any clinical evaluation. Blood samples are taken from each animal via retro-orbital bleeding prior to exposure to ultrasound, then at day five, and after the last exposure, at day ten. To analyze the blood samples a (GhbAlc) ELISA kit
(Cusabio Biotech Co., Ltd, Japan) is used. All data documenting experimental details and study procedures are recorded and analyzed to assess effect on the levels of glycated hemoglobin A1c.


In this example, anti-AGE monoclonal antibody 6D12 (Ando K. et al., supra), or anti-AGE humanized monoclonal antibody is conjugated to a toxin, such as pore-forming toxins or PTFs (Aroian R. et al., Pore-Forming Toxins and Cellular Non-Immune Defenses (CNIDs), Current Opinion in Microbiology, 10:57-61 (2007)), magnetic nanoparticles, magnetic spin-vortex discs (Dobson J., "A Twist on Tumour Targeting," Nature Materials," 9, 95-96 (2010)), or a cytotoxic agent, such as selenocystamine, and IP injected in ZDF rats to selectively bind and remove AGE-modified erythrocytes.

[26] ZDF rats are IP injected in the volume of 200 µl for the initial loading dose of 10 mg/kg of the anti-AGE-monoclonal antibody or with 200 µl PBS 1x control. Each rat receives an IP injection per week for a total of six weeks. The animals are weighed weekly and are observed daily for any clinical evaluation. Blood samples are taken from each animal via retro-orbital bleeding every week. The level of glycated hemoglobin A1c is used as a marker for removal of AGE-modified erythrocytes. All data documenting experimental details and study procedures are recorded and analyzed to assess effect on the levels of glycated hemoglobin A1c.

[27] Example 3 (Prophetic) Removal of AGE-Modified Erythrocytes by Panning Selection

In this example, AGE-modified erythrocytes are isolated from a patient by panning selection, using an anti-AGE monoclonal antibody. Extracorporeal blood purification is utilized to remove AGE-modified cells from a patient.

[29] The patient's blood is passed through an extracorporeal tubing system containing a sorbent agent, i.e. an anti-AGE monoclonal antibody to selectively
remove AGE-modified erythrocytes from the blood. Anti-AGE monoclonal antibodies attached to a solid substrate through their Fc region, bind AGE-modified erythrocytes and remove them from the patient's blood. The blood is recirculated through extracorporeal circulation to remove most AGE-modified erythrocytes and the duration of the procedure is performed following standards known in the art for removing other corpuscolated elements from the blood, e.g. platelets. Gutensohn K. et al., "Extracorporeal Plateletpheresis Induces the Interaction of Activated Platelets with White Blood Cells," Vox Sanguinis, Vol. 78(2), 101-05 (2000). At the end of the procedure, the patient's intracorporeal circulation is restored.

[30] Alternatively, an anti-AGE monoclonal antibody conjugated to a marker, e.g. a fluorescent marker, is injected into a patient. The patient's blood is passed through an extracorporeal tubing system connected to a cell sorter. AGE-modified erythrocytes bound to anti-AGE monoclonal antibodies are sorted by selecting the fluorescent erythrocytes and therefore removed from the patient's blood. At the end of the procedure, the patient's intracorporeal circulation is restored.

[31] Example 4 (Prophetic) Removal of AGE-Modified Erythrocytes by Pore-Forming Toxins (PFTs)

[32] In this example, AGE-modified erythrocytes are targeted by anti-AGE monoclonal antibodies conjugated to a pore-forming toxin. Pore-forming toxins cause osmotic lysis in erythrocytes. Pore-forming toxins can be conjugated to monoclonal antibody to specifically target a particular cell type. See for example, U.S. Patent 5,817,771 , "Cell Targeted Lytic Pore-Forming Agents."

[33] Anti-AGE monoclonal antibodies conjugated to a pore-forming toxin are injected in a patient. The anti-AGE monoclonal antibodies selectively bind and cause the lysis of AGE-modified erythrocytes via the conjugated pore-forming toxin.

[34] Example 5 (Prophetic) Removal of AGE-Modified Cells in Atherosclerotic Lesions by Pore-Forming Toxins (PFTs)
In this example, AGE-modified cells in atherosclerotic lesions are targeted by anti-AGE monoclonal antibody 6D12 (Ando K. et al., supra), or anti-AGE humanized monoclonal antibody conjugated to a toxin, such as pore-forming toxins or PTFs (Aroian R. et al., supra). Pore-forming toxins cause osmotic lysis in AGE-modified cells. Pore-forming toxins can be conjugated to monoclonal antibody to specifically target a particular cell type. See for example, U.S. Patent 5,817,771, "Cell Targeted Lytic Pore-Forming Agents."

Anti-AGE monoclonal antibodies conjugated to a pore-forming toxin are injected in a patient. The anti-AGE monoclonal antibodies selectively bind and cause the lysis of AGE-modified cells in atherosclerotic lesions, such as intima cells, endothelial cells, smooth muscle cells, macrophages, and foam cells, via the conjugated pore-forming toxin.
REFERENCES


What is claimed is:


2. The method of claim 1, wherein the AGE-modified cells are erythrocytes.

3. The method of any of the preceding claims, wherein the AGE-modified cells are at least one cell type selected from the group consisting of intima cells, endothelial cells, smooth muscle cells, macrophages, and foam cells.

4. The method of any of the preceding claims, wherein the patient is a mammal.

5. The method of any of the preceding claims, wherein the patient is a human.

6. The method of any of the preceding claims, wherein the patient has Syndrome-X.

7. The method of any of the preceding claims, wherein the AGE-modified erythrocytes are removed by destroying the AGE-modified erythrocytes with ultrasound.

8. The method of any of the preceding claims, wherein the AGE-modified erythrocytes are removed by binding with an anti-AGE monoclonal antibody.

9. The method of any of the preceding claims, wherein the anti-AGE monoclonal antibody is conjugated to at least one agent selected from the group consisting of a toxin or a cytotoxic agent, magnetic nanoparticles, and magnetic spin-vortex discs.
10. The method of any of the preceding claims, wherein the AGE-modified cells are removed by bind with an anti-AGE monoclonal antibody.

11. The method of any of the preceding claims, wherein the anti-AGE monoclonal antibody is conjugated to at least one agent selected from the group consisting of a toxin or a cytotoxic agent, magnetic nanoparticles, and magnetic spin-vortex discs.

12. A method of removing AGE-modified erythrocytes from blood, comprising damaging or destroying an AGE-modified erythrocyte with ultrasound.


14. The method of any of the preceding claims, wherein the anti-AGE monoclonal antibody is conjugated to an agent selected from the group consisting of a toxin or a cytotoxic agent, magnetic nanoparticles, and magnetic spin-vortex discs.


16. The method of any of the preceding claims, wherein the anti-AGE monoclonal antibody is conjugated to an agent selected from the group consisting of a toxin or a cytotoxic agent, magnetic nanoparticles, and magnetic spin-vortex discs.

17. The method of any of the preceding claims, wherein the AGE-modified cells are removed from atherosclerotic lesions.

18. The method of any of the preceding claims, wherein the AGE-modified cells are at least one cell type selected from the group consisting of intima cells, smooth muscle cells, macrophages and foam cells.

20. The use according to any of the preceding claims, wherein the medicament comprises an effective amount of the anti-AGE antibody, together with at least one pharmaceutically acceptable excipient.

21. The use according to any of the preceding claims, wherein the excipient comprises a diluent, adjuvant or carrier.

22. The use of any of the preceding claims, wherein the medicament is prepared for administration.

23. The use according to any of the preceding claims, wherein the medicament is prepared for administration to a mammal.

24. The use according to any of the preceding claims, wherein the mammal is a human.

25. The use according to any of the preceding claims, wherein the human has Syndrome-X.

26. The use of any of the preceding claims, wherein the anti-AGE monoclonal antibody is conjugated to at least one agent selected from the group consisting of a toxin or a cytotoxic agent, magnetic nanoparticles, and magnetic spin-vortex discs.

27. The use of any of the preceding claims, wherein AGE-modified erythrocytes are removed by binding with the anti-AGE monoclonal antibody.
28. The use of any of the preceding claims, wherein the anti-AGE monoclonal antibody binds and removes AGE-modified cells in atherosclerotic lesions.

29. The use of any of the preceding claims, wherein the AGE-modified cells are at least one cell type selected from the group consisting of intima cells, smooth muscle cells, macrophages and foam cells.

30. The use of any of the preceding claims, wherein the anti-AGE monoclonal antibody is a fluorescent monoclonal antibody.

31. The use of any of the preceding claims, wherein the anti-AGE monoclonal antibody is a humanized monoclonal antibody.


34. A monoclonal antibody according to any of the preceding claims, wherein the patient is a mammal.

35. A monoclonal antibody according to any of the preceding claims, wherein the mammal is a human.

36. A monoclonal antibody according to any of the preceding claims, wherein the patient has Syndrome-X.

37. The monoclonal antibody according to any of the preceding claims, wherein the anti-AGE monoclonal antibody is a humanized monoclonal antibody.
38. The monoclonal antibody according to any of the preceding claims, wherein the anti-AGE monoclonal antibody is a fluorescent monoclonal antibody.

39. The monoclonal antibody according to any of the preceding claims, wherein the anti-AGE monoclonal antibody is conjugated to at least one agent selected from the group consisting of a toxin or a cytotoxic agent, magnetic nanoparticles, and magnetic spin-vortex discs.

40. The monoclonal antibody according to any of the preceding claims, wherein AGE-modified erythrocytes are removed by binding with the anti-AGE monoclonal antibody.

41. The monoclonal antibody according to any of the preceding claims, wherein the anti-AGE monoclonal antibody binds and removes AGE-modified cells in atherosclerotic lesions.

42. A pharmaceutical composition comprising an effective amount of the monoclonal antibody, together with at least one pharmaceutically acceptable excipient.

43. A pharmaceutical composition according to any of the preceding claims, wherein the excipient comprises a diluent, adjuvant or carrier.