EUROPEAN PATENT SPECIFICATION

APOPTOTIC ENTITIES FOR USE IN TREATMENT OF NEURODEGENERATIVE AND OTHER NEUROLOGICAL DISORDERS

APOPTOTISCHE KÖRPER ZUR VERWENDUNG IN DER BEHANDLUNG VON NEURODEGENERATIVEN UND SONSTIGEN NERVENERKRANKUNGEN

ENTITES APOPTOTIQUES S’UTILISANT DANS LE TRAITEMENT DES TROUBLES NEURODEGENERATIFS ET AUTRES TROUBLES NEUROLOGIQUES

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WO-A-00/41705 WO-A-93/15779

• B. BODEY ET AL.: "APOPTOSIS IN THE MAMMALIAN THYMUS DURING NORMAL HISTOGENESIS AND UNDER VARIOUS IN VITRO AND IN VIVO EXPERIMENTAL CONDITIONS." IN VIVO, vol. 12, no. 1, 1998, pages 123-134, XP001064255 Athens, GR

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Description

Field of the Invention

[0001] This invention relates to biochemical and biological compositions and to the uses thereof in the treatment and/or prophylaxis of various neurodegenerative and other neurological disorders in mammalian patients. More particularly, it relates to treatment and prophylaxis of neurodegenerative and other neurological disorders by administration of compositions containing the mammalian cellular materials and fragments thereof, and to the compositions containing the mammalian cellular materials and fragments themselves, and to processes for preparing such compositions.

Background of the Invention

[0002] Two mechanisms of cell death in the body are recognized, necrosis and apoptosis. Apoptosis is the process of programmed cell death, described by Kerr et al in 1992 [Kerr JFR, Wylie AH, Currie AR (1992). "Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. "British Journal of Cancer 26: 239-257"], by which steady-state levels of the various organ systems and tissues in the body are maintained as continuous cell division and differentiation takes place. Cells undergoing apoptosis often exhibit distinctive morphological changes such as a pronounced decrease in cell volume, modification of the cytoskeletons resulting in pronounced membrane blebbing, a condensation of the chromatin, and degradation of the DNA into oligonucleosomal fragments. Following these morphological changes, an apoptotic cell may break up into a number of small fragments known as apoptotic bodies, consisting essentially of membrane-bound bodies containing intact organelles, chromatin etc. Apoptotic bodies are normally rapidly removed from the body by phagocytosis by macrophages, dendritic cells and other antigen presenting cells, before they can become lysed and release their potentially pro-inflammatory intracellular contents.

[0003] In simple outline, apoptosis is thought to proceed as follows. Three phases can be identified in the apoptotic mechanism of programmed cell death:

- Induction phase;
- Effector phase; and
- Degradation phase.

[0004] The induction phase is dependent, in part, on specific interactions of death-inducing signals at the cell surface membrane. One common signal is initiated by the binding of specific ligands to receptors of the TNF receptor family present on the cell membrane. One important such receptor is Fas (APO-1, CD95), which interacts with Fas-ligand to initiate apoptosis.

[0005] The effector phase, activated by the binding of receptors and ligands of the induction phase, leads to the activation of caspases, cysteinyl-aspartate-requiring proteinases (proteolytic enzymes), including caspases 1 and 8. This activation may be associated with a change in the permeability of mitochondria, allowing the release of cytochrome-c which is involved in caspase activation. Activated caspases initiate a chain of lethal proteolytic events culminating in the changes in chromatin and cytoskeletal components seen in apoptosis.

[0006] Many cells undergoing apoptosis can be identified by a characteristic ‘laddering’ of DNA seen on agarose gel electrophoresis, resulting from cleavage of DNA into a series of fragments. These changes occur a few hours before death of the cell as defined by the ability of a cell to exclude vital dyes. The appearance of DNA laddering on agarose gel electrophoresis follows extraction of DNA from cells is one recognised method of identification of apoptosis in cells [Loo, D.T. and Rillema, J.R. (1998) "Measurement of Cell Death." Methods in Cell Biology 57: 251-264], although it is not always sensitive enough to detect apoptosis. In situ labelling of nuclear DNA fragmentation, for example, using commercially available terminal dUTP nick end labelling (TUNEL) assays, are an alternative and more reproducible measure for the determination of fragmented DNA in apoptotic cells and cells undergoing apoptosis [Gavrieli Y, Sherman Y, Ben-Sasson SA (1992)" Identification of programmed cell death in situ via specific labelling of nuclear DNA fragmentation". Journal of Cell Biology 119: 493-501].


A number of other methods of identification of cells undergoing apoptosis and of apoptotic cells, many using monoclonal antibodies against specific markers for apoptotic cells, have also been described in the scientific literature.

Necrosis, in contrast, is cell death of a pathological nature, resulting from injury, bacterial toxin effects, inflammatory mediators, etc., and involving membrane rupture and release of intracellular contents to the surrounding tissue, often with harmful inflammatory consequences. Accordingly, one of the ways in which necrotic cells may be detected and characterized is by detection of compromised cell membranes, e.g. by methods of staining with propidium iodide followed by flow cytometry or microscopy.

In a first aspect of the present invention there is provided the use of apoptotic bodies and/or apoptotic cells in the preparation of a medicament for the treatment and/or prophylaxis of a neurodegenerative or neurological disorder in a mammalian patient, the apoptotic bodies and/or apoptotic cells being compatible with the blood cells of the patient.

In a second aspect of the present invention there is provided a pharmaceutical composition comprising a liquid suspension of cellular material, from 10% to 90% of said cellular material being apoptotic bodies and/or apoptotic cells.

This invention is directed, in part, to the novel compositions, which comprise a liquid suspension of cellular material, from 10% to 90% of said cellular material being apoptotic bodies and/or apoptotic cells, and/or prophylaxis of neurodegenerative and/or other neurological disorders by the administration of apoptotic cells and/or bodies.

Neurodegenerative disorders, including Down’s syndrome, Alzheimer’s disease and Parkinson’s disease, are associated with increased levels of reactive oxygen species (ROS), certain inflammatory cytokines, including interleukin-1β (IL-1β) [see Griffin WST, Stanley LC, Ling C, White L, Macleod V. Perrot LJ, White OL, Aroz C (1989). Brain interleukin 1 and S-100 immunoreactivity are elevated in Down’s syndrome and Alzheimer disease. Proceedings of the National Academy of Sciences USA 867611-7615; Mogi M, Harada M, Nara-bayashi H, Inagaki H, Minami M, Nagatsu T (1996). Interleukin (IL)-1 beta, IL-1, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson’s disease. Neuroscience Letters 211:13-16]. It has also been shown that IL-1β inhibits long-term potentiation in the hippocampus [Murray CA, Lynch MA (1998). Evidence that increase hippocampal expression of the cytokine interleukin-1β is a common trigger for age and stress-induced impairments in long-term potentiation. Journal of Neuroscience 18:2974-2981]. Long-term potentiation in the hippocampus is a form of synaptic plasticity and is generally considered to be an appropriate model for memory and learning [Bliss TVP, Collinridge GL. (1993). A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361:31-39]. Thus, inappropriate cytokine expression in the brain is currently believed to be involved in the development and progression of neuroinflammatory diseases.

Neurodegenerative and other neurological disorders treatable by the present invention include Down’s syndrome, Alzheimer’s disease, Parkinson’s disease, senile dementia, depression and the like. In summary, it can be substantially any neurodegenerative or other neurological disorder.

“Apoptotic cells” and "apoptotic bodies," as the terms are used herein, means cells and cell bodies which exhibit one or more of the following apoptosis-characterizing features: surface exposure of phosphatidylserine, as detected by standard, accepted methods of detection such as Annexin V staining; alterations in mitochondrial membrane permeability measured by standard, accepted methods (e.g. Salvioi, S., Ardizzoni, A., Franceschi, C. Cossarizza, A. (1997) "JC-1, but not DiOC6(3) or Rhodamine 123, is a Reliable Fluorescent Probe to assess Delta Psi Changes in Intact Cells: Implications for Studies on Mitochondrial Functionality during Apoptosis," FEBS Letters 411: 77-82]; evidence of DNA fragmentation such as the appearance of DNA laddering on agarose gel electrophoresis following extraction of DNA
The apoptotic cells and/or apoptotic bodies for use in the present invention preferably comprise not more than about 35 weight percent of necrotic cells and/or necrotic bodies based on the total weight of the apoptotic cells/bodies and necrotic cells/bodies; more preferably, not more than about 20 weight percent; and even more preferably, not more than about 10 weight percent. At these levels, the presence of such necrotic cells and/or bodies is believed not to significantly alter in vivo processes. In its most preferred embodiment, the apoptotic cells/bodies are substantially free of necrotic cells and/or bodies (i.e., less than about 2 weight percent of necrotic cells/bodies).

A variety of methods of inducing apoptosis in mammalian cells, so as to create apoptotic cells and apoptotic bodies, are known in the art and essentially any of these can be adopted in preparing apoptotic bodies for use in the present invention. One such method is the subjecting of the cells to ionizing radiation (γ-rays, x-rays, etc.) and/or non-ionizing electromagnetic radiation including ultraviolet light. Apoptosis can be induced by subjecting cells to ultrasound.


Yet another method is the application of oxidative stress to cells extracorporeally (see for example Buttke and Sandstrom (1994) "Oxidative Stress as a Mediator of Apoptosis," Immunology Today, Vol. 15:7-10). This can be achieved by treating the cells, in suspension, with chemical oxidizing agents such as hydrogen peroxide, other peroxides and hydroperoxides, ozone, permanganates, periodates, and the like. Biologically acceptable such oxidizing agents are preferably used, so as to reduce potential problems associated with residues and contaminations of the apoptotic cells and apoptotic bodies so formed.

The present invention is not restricted to any particular method of producing apoptotic cells and apoptotic bodies, for use herein, and any suitable, known process can be used.

Methods for the detection and quantitation of apoptosis can be used to determine the presence and level of apoptosis in the preparation to be administered to the patient in the present invention. At least one of the methods from those described in the introduction above should be used to confirm the level of apoptosis achieved prior to administration. They are suitably purified prior to use, by methods known in the art, such as differential centrifugation.

In preparing the apoptotic cells and/or apoptotic bodies, care should be taken not to apply excessive levels of oxidative stress, radiation, drug treatment, etc., since otherwise there is a significant risk of causing necrosis of at least some of the cells under treatment. Necrosis causes cell membrane rupture and the release of cellular contents often with biologically harmful results, particularly inflammatory events, so that the presence of necrotic cells and their components along with the apoptotic bodies is best avoided. Appropriate levels of treatment of the cells to create apoptotic bodies for use in the present invention depend to some extent on the nature of the chosen cells and cellular composition, and the type of treatment chosen to induce apoptosis. Such appropriate levels are readily determinable by those skilled in the art, having regard to the available scientific literature on the subject including the above-reference articles.

One preferred process according to the present invention involves the culture of cells from the patient, or a compatible mammalian cell line. The cultured cells may then be treated to induce apoptosis and to create apoptotic cells and/or apoptotic bodies therein. The cells, suspended in the patient’s plasma or another suitable sus-
pension medium, such as saline or a balanced mammalian cell culture medium, can then be administered as indicated below. The numbers of apoptotic cells and/or bodies can be determined by published methods available in the scientific literature on the subject including the above-reference articles. The numbers of such apoptotic cells and/or apoptotic bodies required for administration to the patient to obtain the required clinical benefit will vary depending on the source of cells, the patient’s condition, the age and weight of the patient and other relevant factors which are readily determinable by the attending clinician.

[0032] Thus, an example of a preferred process according to the present invention accordingly involves extraction of an aliquot of blood from the patient to be treated, separation of the white cells therefrom, and treatment of the white cells under apoptosis-causing conditions, so as to create a cellular composition in which significant numbers of the white cells therein have been apoptosed so as to create therein substantial numbers of apoptotic cells or bodies. Then the treated composition is administered to the patient. More preferably, T lymphocytes, isolated from the blood by known means, and suspended as above, may be used as a source of apoptotic cells and apoptotic bodies.

[0033] The number of viable cells selected for treatment to create apoptotic cells and/or apoptotic bodies is suitably up to about 4 x 10^9, preferably from about 1,000,000 to about 1,000,000,000 and most preferably from about 50,000,000 to about 150,000,000, for each administration to a human patient. From about 10% to 90%, preferably from about 30% to 70% of the cellular composition for administration is comprised of apoptotic bodies and/or apoptotic cells, the balance being viable cells and necrotic cells. Accordingly, the preferred amounts of apoptotic cells and/or apoptotic bodies for administration are those produced by subjecting these numbers of cells to the apoptosing conditions. When whole blood is used as the source of the cells to be subjected to the apoptosis inducing conditions, these numbers of white cells are obtainable in blood aliquots of volume up to about 400 ml, preferably up to 100 ml. More specifically, 50,000,000 - 150,000,000 cells is equivalent to the white cells in blood aliquots of volume 10 - 30 ml.

[0034] The volume of the aliquot of blood withdrawn from the patient for treatment to create apoptotic cells and/or apoptotic bodies therein is suitable up to about 400 ml, preferably from about 0.1 to about 100 ml and most preferably from about 5 to about 15 ml. Accordingly, the preferred amounts of apoptotic cells and/or apoptotic bodies for administration are those corresponding to the numbers derivable from the white blood cells, or isolated T lymphocytes, contained in such quantities of whole blood, following subject to apoptosis-inducing conditions.

[0035] The suspension of treated apoptotic cells and/or bodies for administration to the patient is prepared in a biologically acceptable liquid suspending medium, such as the patient’s serum or plasma, saline or balanced mammalian cell culture medium. The addition of other factors, such as cytokines, hormones, products of stressed cells or other appropriate biologically active material may enhance the benefit of the administered apoptotic cellular materials. The aliquot can be introduced into the patient’s body by any suitable method, most preferably intramuscular injection but also including subcutaneous injection, mini-grafting, intra-peritoneal injection, intra-arterial injection, intravenous injection and oral administration. The apoptotic entities can be delivered to the specific body organ and/or site by using any appropriate, known delivery system.

[0036] The compositions of this invention may optionally include a pharmaceutically acceptable excipient. Some examples of suitable excipients include sterile water, sterile saline, phosphate buffered saline, and the like.

[0037] When administered, the pharmaceutical compositions comprise an effective amount of apoptotic bodies/cells to induce a suitable prophylactic and/or therapeutic response in the patient at risk of suffering or suffering from a neurodegenerative disease. Preferably, the composition administered to the mammalian patient comprises from about 10,000 to 10,000,000 apoptotic cells or bodies per kilogram of body weight, more preferably from about 500,000 to 5,000,000 and most preferably from about 1,500,000 to about 4,000,000 apoptotic cells and/or bodies per kg body weight. The specific dose employed will, of course, be dependent upon the age, weight and severity of the disease in the treated patient all of which are within the skill of the attending clinician.

[0038] For most effective treatment and/or prophylaxis of mammalian disorders involving a neurodegenerative or neurological disorder, the patient may be given a course of treatments with apoptotic cells and/or bodies according to the invention. Each course of treatment may involve administration to the patient of from 1 to 6 aliquots of suspended cellular material, as described above. No more than one such aliquot should be administered per day, and the maximum rest period between any two consecutive administrations should be not greater than about 21 days. Booster treatments as described below may advantageously be used. To maintain the desired effects, the patient may undergo booster treatments, with a further course of administration of aliquots of suspended apoptotic cells and/or apoptotic bodies as described above, at intervals of three to four months.

[0039] As noted, the present invention is applicable to the treatment and prophylaxis of a wide variety of mammalian neurodegenerative and other neurological disorders. These include, but are not limited to, Down’s Syndrome, Alzheimer’s disease, Parkinson’s disease, senile dementia, depression, multiple sclerosis, Huntington’s disease, peripheral neuropathies, spinal cord diseases, neuropathic joint diseases, chronic inflammatory demyelinating disease (CIDP), neuropathies including mononeuropathy, polyneuropathy, symmetrical distal sensory
nerupathy, cystic fibrosis, neuromuscular junction disorders and myasthenias. In summary, it can be substan-
tially any neurodegenerative or other neurological disor-
der.

[0040] The invention is further described, for illustra-
tive purposes, in the following specific examples.

EXAMPLE 1

[0041] Experiments to demonstrate the invention were conducted on laboratory mice, under approved condi-
tions for conducting such experiments.

[0042] The effectiveness of the treatment according to a preferred embodiment of the present invention, on con-
tact hypersensitivity (CHS), an example of a Th-1 cell
flammatory disorder which is known to be mediated by
flammatory cytokines, was assessed on laboratory
ice, according to approved animal experimentation pro-
dcedures, using the method described by Kondo et. al.,
"Lymphocyte function associated antigen-1 (LFA-1) is re-
quired for maximum elicitation of allergic contact dema-
titis" Br. J. Dermatol. 131:354-359 (1994), with minor var-
ations. The disclosure thereof is incorporated herein by
reference. Briefly, to induce CHS, the abdominal skin of
each mouse was shaved and painted with dinitrofluor-
obenzene DNFB, the sensitizing chemical, using 25 µl
of 0.5% DNFB in acetone:olive oil solution. This sen-
sitization was applied to two groups of Balb/c mice, 10
animals in total.

[0043] Apoptotic bodies were prepared from murine
fibroblasts. The murine fibroblasts were treated with 50
mM sodium butyrate in RPMI medium, at confluency for
one day, and then the sodium butyrate medium was
changed. To increase the number of apoptotic cells and
bodies, the cells can additionally be irradiated with U
light (e.g. 75 mj). Supernatant containing floating cells is
removed 24 hours following irradiation.

[0044] Apoptotic bodies were quantitatively by centrifug-
ing the supernatant (1200 rpm, 5 minutes), aspirating the
supernatant, washing the resulting cell pellet with PBS
and centrifuging again, as above. The pellet containing
the apoptotic bodies was re-suspended in PBS. The cells
were stored in PBS at 4°C for the duration of the exper-
iment. The cells to be stained for quantitation were re-
suspended in 1X binding buffer at a concentration of
1×10^6 cells/ml. 100 µl of the cells were transferred to a
5 ml tube, and 10 µl of fluorescein-conjugated annexin
V and 10 µl propidium iodide reagent was added. The
cells were gently vortexed and the cell mixture incubated
for 15 minutes at 25°C in the dark. Following the incuba-
tion, 400 µl of 1X binding buffer is added to each tube.
The sample was analyzed on a flow cytometer over one
hour.

[0045] Of the two groups of sensitized mice, the first,
control group A, received no treatment. The second, test
group B, was treated with an injection of suspended ap-
optotic bodies prepared as described above, 50 µl vol-
ume containing at least 150,000 bodies per injection of
blood subjected to stressors as described above. Treat-
ments, each involving intramuscular injection of 50 µl of
the respective liquid, started on the day of sensitization,
and were repeated every day for a total of six days. On
the same day as the last treatment, but after its admin-
istration, the animals were challenged with DNFB, by ap-
plying to the right ear of each animal 10µl of 0.2% solution
of DNFB in acetone and olive oil. To the left ear of each
animal was applied the acetone/olive oil solvent, without
DNFB. Inflammation due to CHS manifests itself in a
swelling of the right ears. Ear thickness was measured,
24 hours after challenge, with a Peacock spring-loaded
micrometer (Ozaki Co., Tokyo, Japan). The results were
expressed as the thickness and difference in thickness
of the right ears and the left ears of each animal, at 24
hours after challenge.

[0046] The experiments were repeated, using more
sets of two groups of animals, a sufficient number of times
to ensure statistical significance in the results. A notable
and significant reduction in ear thickness (inflammation)
was observed with the animals treated with the apoptotic
cells and apoptotic bodies suspension in accordance with
the invention, as compared with the untreated group,
demonstrating a significant reduction in inflammation.
The results are presented in the following Table, and on
the accompanying Figure, as a bar graph of net ear swel-
ding (difference between right ear and left ear thickness),
for each group, with "standard deviation" shown by the
vertical line at the top of each column.

<table>
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<th>Difference</th>
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<td>14</td>
</tr>
<tr>
<td>A</td>
<td>18</td>
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</tr>
<tr>
<td>A</td>
<td>18</td>
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</tr>
<tr>
<td>A</td>
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</tr>
<tr>
<td>Mean</td>
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<tr>
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<th>Right ear</th>
<th>Difference</th>
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</table>

[0047] An analysis of the suspension of apoptotic cells
and bodies administered to the animals of test group B
indicated the presence therein of approximately 40% ap-
optotic cells and bodies, balance viable cells and minor
amounts of necrotic cells (not more than 20%), the presence of which is believed to be insignificant in the in vivo process.

**EXAMPLE 2**

[0048] The above test procedure was repeated on similar groups of animals, a control group and a test group, but using a suspension of apoptotic cells and bodies on the test group which comprised about 60% apoptotic cells and bodies, balance viable cells and a minor amount (not more than 20%) of necrotic cells. Essentially similar results were obtained.

[0049] The effectiveness of the processes and compositions of the present invention in preventing and alleviating inflammation due to CHS indicates that administration of apoptotic cells and bodies as described up-regulates the in vivo generation of anti-inflammatory Th-2 derived cytokines such as IL-10 (known to be implicated in CHS - see Kondo, McKenzie and Sauder, “The Journal of Investigative Dermatology,” Vol. 103, 1994, page 811-814) and/or down-regulates Th-1 inflammatory cytokines such as TNFα and IL-6. These inflammatory cytokines are implicated in inflammation-related disorders of the brain, namely the neuroinflammatory, neurodegenerative and neurological disorders such as Alzheimer’s disease, senile dementia, multiple sclerosis, depression, Down’s syndrome, Huntington’s disease, peripheral neuropathies, spinal cord diseases, neuropathic joint diseases, chronic inflammatory demyelinating disease (CIDP), neuropathies including mononeuropathy, polynuropathy, symmetrical distal sensory neuropathy, cystic fibrosis, neuromuscular junction disorders, myasthenias and Parkinson’s disease.

[0050] Neurodegenerative diseases, including Down’s syndrome, Alzheimer’s disease and Parkinson’s disease, are associated with increased levels of certain inflammatory cytokines, including interleukin-1β (IL-1β) [see Griffin WST, Stanley LC, Ling C, White L, Macleod V, Perrot LJ, White CL, Araoz C (1989). Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proceedings of the National Academy of Sciences USA 867611-7615; Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T (1996). Interleukin (IL)-1 beta, IL-1, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson’s disease. Neuroscience Letters 211:13-16]. It has also been shown that IL-1β inhibits long-term potentiation in the hippocampus [Murray CA, Lynch MA (1998). Evidence that increase hippocampal expression of the cytokine interleukin-1β is a common trigger for age and stress-induced impairments in long-term potentiation. Journal of Neuroscience 18:2974-2981]. Long-term potentiation in the hippocampus is a form of synaptic plasticity and is generally considered to be an appropriate model for memory and learning [Bliss TVP, Collinridge GL, (1993). A synaptic model of memory: long-term po-tentiation in the hippocampus, Nature 361:31-39]. Thus, inappropriate cytokine expression in the brain is currently believed to be involved in the development and progression of neurodegenerative diseases. Consequently, the finding of success in CHS treatment reported in the above Examples, with its attendant down-regulation of Th-1 inflammatory cytokines, is indicative of successful use of the process and compositions in the treatment and prophylaxis of a wide variety of neurological disorders including those discussed above.

**Claims**

1. The use of apoptotic bodies and/or apoptotic cells in the preparation of a medicament for the treatment and prophylaxis of a neurodegenerative or neurological disorder in a mammalian patient, the apoptotic bodies and/or apoptotic cells being compatible with the blood cells of the patient.

2. Use as in claim 1 wherein the medicament is a liquid suspension.

3. Use as in claim 1 or 2, wherein the apoptotic bodies and/or apoptotic cells constitute from 10% to 90% of the cellular portion of the medicament.

4. Use as in claim 3 wherein the apoptotic bodies and/or apoptotic cells constitute from 30% - 70% of the cellular portion of the medicament.

5. Use according to any preceding claim wherein the apoptotic bodies and/or apoptotic cells are derived from extracorporeal treatment of blood cells compatible with those of the mammalian patient.

6. Use according to any of claims 1-4 wherein the apoptotic bodies and/or cells are derived from established cultured cell lines.

7. Use according to claim 5 wherein the blood cells are white cells.

8. Use according to claim 7 wherein the white blood cells are the patient’s own white blood cells.

9. Use according to claim 8 wherein the blood cells are the patient’s own T-lymphocytes.

10. Use according to any preceding claim wherein the disorder is selected from the group consisting of Alzheimer’s disease. Senile dementia, multiple sclerosis, Parkinson’s disease, depression, Down’s syndrome, Huntington’s disease, peripheral neuropathies, spinal cord diseases, neuropathic joint diseases, chronic demyelinating disease (CIDP), neuropathies including mononeuropathy, polynuropathy,
11. Use according to any preceding claim in preparation of a unit dosage of medicament for administration to a human patient, said dosage containing from 10,000 to 10,000,000 apoptotic bodies and/or apoptotic cells per kilogram body weight of the patient.

12. Use according to claim 11 wherein said dosage contains from 500,000 to 5,000,000 apoptotic bodies and/or apoptotic cells per kilogram body weight of the patient.

13. Use according to claim 11 wherein said dosage contains from 1,500,000 to 4,000,000 apoptotic bodies and/or apoptotic cells per kilogram body weight of the patient.

14. The use of any one of the preceding claims wherein the medicament is suitable for administration by intramuscular injection.

15. A pharmaceutical composition comprising a liquid suspension of cellular material, from 10% to 90% of said cellular material being apoptotic bodies and/or apoptotic cells.

16. A pharmaceutical composition according to claim 15 wherein the apoptotic bodies and/or apoptotic cells comprise from 30% - 70% of the cellular material.

Patentansprüche


2. Verwendung nach Anspruch 1, dadurch gekennzeichnet, dass das Medikament eine flüssige Aufschlammung ist.

3. Verwendung nach Anspruch 1 oder 2, dadurch gekennzeichnet, dass die apoptotischen Körper und/oder apoptotischen Zellen 10-90% des zellulären Teils des Medikaments bilden.


5. Verwendung nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, dass die apoptotischen Körper und/oder apoptotischen Zellen von einer außerkorpérlichen Behandlung der Blutzellen abgeleitet sind, die mit denen des Säugetierpatienten kompatibel sind.


7. Verwendung nach Anspruch 5, dadurch gekennzeichnet, dass die Blutzellen weiße Zellen sind.

8. Verwendung nach Anspruch 7, dadurch gekennzeichnet, dass die weißen Blutzellen die eigenen, weißen Blutzellen des Patienten sind.

9. Verwendung nach Anspruch 8, dadurch gekennzeichnet, dass die weißen Blutzellen die eigenen T-Lymphozyten des Patienten sind.

10. Verwendung nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, dass die Störung aus der Gruppe ausgewählt ist, die die Alzheimer-Erkrankung, senile Dementz, multiple Sklerose, Parkinson-Krankheit, Depression, das Down-Syndrom, die Huntington-Krankheit, periphere Neuropathien, Rückenmarkerscheinungen, Erkrankungen neuropathischer Verbindungen, die chronische, demyelinierende Erkrankung (CIPD) und Neuropathien umfasst, die die Mononeuropathie, Polyneuropathie, symmetrische, distale, sensorische Neuropathie, zystische Fibrose, neuromuskuläre Verbindungskrankheit und Myasthenien einschließen.


12. Verwendung nach Anspruch 11,
dadurch gekennzeichnet,
dass die Einheitsdosis 500000 bis 5000000 apoptotische Körper und/oder apoptotische Zellen je Kilogramm des Körpergewichts des Patienten enthält.

13. Verwendung nach Anspruch 11,
dadurch gekennzeichnet,
dass die Einheitsdosis 1500000 bis 4000000 apoptotische Körper und/oder apoptotische Zellen je Kilogramm des Körpergewichts des Patienten enthält.

14. Verwendung nach einem der vorhergehenden Ansprüche,
dadurch gekennzeichnet,
dass das Medikament für die Verabreichung durch eine intramuskuläre Injektion geeignet ist.


16. Pharmazeutische Zusammensetzung nach Anspruch 15,
dadurch gekennzeichnet,
dass die apoptotischen Körper und/oder apoptotischen Zellen 30-70% des Zellmaterials enthalten.

Revendications

1. Utilisation de corps apoptotiques et/ou de cellules apoptotiques dans la préparation d’un médicament destiné au traitement et/ou à la prophylaxie d’un trouble neurodégénératif ou neurologique chez un patient mammifère, les corps apoptotiques et/ou les cellules apoptotiques étant compatibles avec les globules du patient.

2. Utilisation selon la revendication 1, dans laquelle le médicament est une suspension liquide.

3. Utilisation selon la revendication 1 ou 2, dans laquelle les corps apoptotiques et/ou les cellules apoptotiques constituent de 10 % à 90 % de la fraction cellulaire du médicament.

4. Utilisation selon la revendication 3, dans laquelle les corps apoptotiques et/ou les cellules apoptotiques constituent de 30 % à 70 % de la fraction cellulaire du médicament.

5. Utilisation selon l’une quelconque des revendications précédentes, dans laquelle les corps apoptotiques et/ou les cellules apoptotiques sont dérivé(e)s d’un traitement extracorporel de globules compatibles avec ceux du patient mammifère.

6. Utilisation selon l’une quelconque des revendications 1 à 4, dans laquelle les corps apoptotiques et/ou les cellules apoptotiques sont dérivé(e)s de lignées cellulaires établies cultivées.

7. Utilisation selon la revendication 5, dans laquelle les globules sont des globules blancs.

8. Utilisation selon la revendication 7, dans laquelle les globules blancs sont les propres globules blancs du patient.

9. Utilisation selon la revendication 8, dans laquelle les globules sont les propres lymphocytes T du patient.

10. Utilisation selon l’une quelconque des revendications précédentes, dans laquelle le trouble est choisi dans le groupe constitué par la maladie d’Alzheimer, la démence sénile, la sclérose en plaques, la maladie de Parkinson, la dépression, le syndrome de Down, la chorée de Huntington, les neuropathies périphériques, les maladies de la moelle épinière, les maladies articulaires neuropathiques, une maladie démyélinisante chronique (MDIC) les neuropathies comprenant une mononeuropathie, une polynéuropathie, une neuropathie sensorielle distale et symétrique, la mucoviscidose, les troubles des jonctions neuromusculaires et les myasthénies.

11. Utilisation selon l’une quelconque des revendications précédentes dans la préparation d’une dose unitaire de médicament destinée à être administrée à un patient humain, ladite dose contenant de 10 000 à 10 000 000 de corps apoptotiques et/ou de cellules apoptotiques par kilogramme de poids corporel du patient.

12. Utilisation selon la revendication 11, dans laquelle ladite dose contient de 500 000 à 5 000 000 de corps apoptotiques et/ou de cellules apoptotiques par kilogramme de poids corporel du patient.

13. Utilisation selon la revendication 11, dans laquelle ladite dose contient de 1 500 000 à 4 000 000 de corps apoptotiques et/ou de cellules apoptotiques par kilogramme de poids corporel du patient.

14. Utilisation selon l’une quelconque des revendications précédentes, dans laquelle le médicament est adapté pour une administration par injection intramusculaire.

15. Composition pharmaceutique comprenant une suspension liquide de matériel cellulaire, de 10 % à 90 % dudit matériel cellulaire étant des corps apoptotiques et/ou des cellules apoptotiques.

16. Composition pharmaceutique selon la revendication
15, dans laquelle les corps apoptotiques et/ou les cellules apoptotiques constituent de 30 % à 70 % du matériel cellulaire.
REFERENCES CITED IN THE DESCRIPTION

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