**Title:** GALACTOSYLCERAMIDE, GLUCOSYLCERAMIDE, LACTOSYLCERAMIDE, AND SPECIFIC CATCHERS THEREFOR FOR USE IN THE PROPHYLAXIS OR THERAPY OF PREDIABETES, DIABETES AND/OR ASSOCIATED COMPLICATIONS

**Abstract**

The use of glycolipids, in particular galactosylceramide, glucosylceramide and lactosylceramide, and specific catchers therefor (antibodies or lectins), in particular monoclonal antibodies, for use in the prophylaxis or therapy of prediabetes, diabetes and/or associated complications in an individual and for use in the production of pharmaceutical preparations for treatment of said conditions is disclosed.
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GALACTOSYLDERAMIDE, GLUCOSYLDERAMIDE,
LACTOSYLDERAMIDE, AND SPECIFIC CATCHERS THEREFOR FOR
USE IN THE PROPHYLAXIS OR THERAPY OF PREDIABETES,
DIABETES AND/OR ASSOCIATED COMPLICATIONS

Technical field of the invention
The present invention relates to the use of glycol-
lipids and specific catchers therefore in treatment of
diabetes.

Background of the invention
Galactosylceramide is a glycolipid consisting of ce-
ramide to which galactose is attached. It is made by the
enzyme ceramide galactosyltransferase which binds its two
parts together. Galactosylceramide is a precursor of sul-
fatide and is present in the neural system and in islets
of Langerhans in small amounts. A sulfatransferase enzyme
is able to attach sulfate to the galactose group and
thereby to convert galactosylceramide to sulfatide. It
is a possibility that galactosylceramide given in vivo is
converted to sulfatide. However, galactosylceramide may
also act by itself and, indeed effects of galactosyl-
ceramide have been described in vitro (Buschard, K., Dia-
mant, M., Bovin, L. F., Fredman, P., Bendtzen, K., Sul-
phatide and its precursor, galactosylceramide, influence
the production of cytokines in human mononuclear cells.
APMIS 104: 938-944, 1996). It has been shown that galac-
tosylceramide can modulate and mainly enhance the produc-
tion of different cytokines from both monocytes and T-
cells after stimulation with LPS and PHA, respectively.
Most importantly TNF and IL-6 production is increased
compared to incubation with LPS and PHA without galacto-
sylceramide.

Glucosylceramide and lactosylceramide are related
glycolipids which likely have the same effects as galac-
tosylceramide.
Summary of the invention

The present invention relates to the use of a glycolipid or a specific catcher thereof for the production of a pharmaceutical preparation for treatment of prediabetes, diabetes and/or associated complications in an individual.

Furthermore, the invention relates to glycolipids, in particular galactosylceramide, glucosylceramide and lactosylceramide, and specific catchers therefore (antibodies or lectins) for use in the prophylaxis or therapy of prediabetes, diabetes and/or associated complications in an individual.

The invention also relates to a specific catcher for the glycolipids according to the invention, said catcher being a monoclonal antibody against galactosylceramide, glucosylceramide or lactosylceramide.

The invention also relates to a method for preventing the development of prediabetes, diabetes and/or associated complications in an individual, wherein a glycolipid, in particular galactosylceramide, glucosylceramide or lactosylceramide, is administered to said individual, preferably at its perinatal stage.

The characterising features or the invention will be evident from the following description and the appended claims.

Detailed description of the invention

As stated above, the invention relates glycolipids, in particular galactosylceramide, glucosylceramide and lactosylceramide, and specific catchers therefore (antibodies or lectins) for use in the prophylaxis or therapy of prediabetes, diabetes and/or associated complications in an individual, as well as to the use of a glycolipid or a specific catcher thereof for the production of a pharmaceutical preparation for treatment of
prediabetes, diabetes and/or associated complications in an individual.

The expression "treatment" used herein relates to both the prophylaxis of said conditions in an individual being in risk of developing any of the conditions and to the therapeutic treatment of an individual who have already developed any of the conditions.

The prophylactic treatment is performed by inducing tolerance to the antigenic glycolipids. When the glycolipids, the specific catchers therefore and/or the pharmaceutical preparation according to the invention is used for this purpose they are preferably administered perinatally to said individual.

The glycolipid used according to the invention is preferably galactosylceramide, glucosylceramide or lactosylceramide. The specific catcher used according to the invention is preferably an antibody or a lectin, and more preferably a monoclonal antibody against galactosylceramide, glucosylceramide or lactosylceramide.

The glycolipid, the specific catcher or the pharmaceutical preparation according to the invention may be administered in any suitable way known to the man skilled in the art. Preferably, they are administered nasally, orally, subcutaneously, intramuscularly, or intravenously.

The glycolipids or the pharmaceutical preparation may lead to increased levels of suppressor or regulator cells or antibodies against lymphocytes recognising the antigenic glycolipids in said individual. Alternatively, they may lead to the removal of antibodies and/or lymphocytes recognising the antigenic glycolipids from the bloodstream of the individual.

It may be suitable to administer the glycolipids according to the invention together with bacterial adjuvants. The pharmaceutical composition according to the invention may therefore also comprises a least one bacte-
rial adjuvant, such as cholera, staphyloccoc or galacto-
sylceramide (alpha-form) of bacterial origin.

The pharmaceutical preparation according to the in-
vention may also comprise substances used to facilitate
the production of the pharmaceutical preparation or the
administration of the preparations. Such substances are
well known to people skilled in the art and may for exam-
pole be pharmaceutically acceptable adjuvants, carriers
and preservatives.

When antibodies are used according to the invention
they will lead to an increase of anti-antibodies in said
individual.

The invention also relates to a method for prevent-
ing the development of prediabetes, diabetes and/or asso-
ciated complications in an individual, wherein a glycol-
lipid, in particular galactosylceramide, glucosylceramide
or lactosylceramide, is administered to said individual,
preferably at its perinatal stage.

The method may be performed by removing lymphocytes
from the individual, contact the lymphocytes with a gly-
colipid, in particular galactosylceramide, glucosylcera-
mide or lactosylceramide, in vitro to make them recognise
this antigen, irradiating them to inhibit their cytoto-
oxicity, and (a) returning them to the individual to raise
suppressor or regulator cells or antibodies against lym-
phocytes reactive with this antigen, or (b) administering
them parenterally to another mammal in order to raise an-
tibodies against lymphocytes reactive with this antigen
in said mammal and then isolating serum containing the
antibodies from said mammal and administering it to the
individual.

The method may also be performed by contacting the
blood stream of the individual with an immobilised gly-
colipid, in particular galactosylceramide, glucosylcera-
mide or lactosylceramide, to remove antibodies and/or
lymphocytes recognising the antigenic glycolipids from
the individual.
Finally, it is also possible to perform the method by parenterally administer an antibody against glycolipids, in particular galactosylceramide, glucosylceramide or lactosylceramide, (a) to said individual in a sufficient amount to raise anti-antibodies in said individual, or (b) to another mammal in order to raise anti-antibodies in said mammal and then isolating serum containing the anti-antibodies from said mammal and administering it to the individual.

The invention will now be further explained in the following example. This example is only intended to illustrate the invention and should in no way be considered to limit the scope of the invention.

Example - Treatment of NOD mice with the intrathymic injections of galactosylceramide in order to modulate the later diabetes incidence

Materials and methods

The NOD mouse model is an animal model of type 1 diabetes. The mice develop spontaneously diabetes within 200 days of life with an incidence of 50% or more. In the present study the animals were examined daily and once a week checked for glucosuria. They were diagnosed as diabetic if their blood glucose values was higher than 200 mg glucose per 100 ml. When diagnosed as diabetics the animals were sacrificed. Otherwise the remaining non-diabetic animals were sacrificed at the end 5 of the study after 200 days of age.

Four groups of animals each containing between 30 and 40 animals were investigated. The animals were injected intrathymically when 3 weeks old. There was injected 50 µl galactosylceramide in 100 µl vehicle (PBS). The control mice were treated with the 100 µl PBS alone. In another experiment liposome preparation of galactosylceramide was made using phosphatidylcholin and galactosylceramide. The control group was treated with phosphatidylcholin and PBS alone.
Results

In the first study with treatment of galactosylceramide in pure form 17 of 37 mice (=45.9%) developed diabetes, whereas 21 of 37 (=56.8%) of the control (PBS-treated) mice developed the disease. In the second study with galactosylceramide in liposome form 14 of 33 (=42.4%) of the galactosylceramide liposome treated mice developed diabetes whereas 18 of 31 (=58.1%) of the control (PBS-treated) mice develop the disease.

Taking the two studies together 31 of 70 (=44.3%) of the galactosylceramide treated mice developed diabetes whereas 39 of 68 (=57.4%) of PBS mice developed the disease. In the first study the diabetes development in the galactosylceramide treated group occurred later than among the control mice.
CLAIMS

1. Use of a glycolipid or a specific catcher thereof for the production of a pharmaceutical preparation for treatment of prediabetes, diabetes and/or associated complications in an individual.

2. Use according to claim 1, wherein said glycolipid is galactosylceramide, glucosylceramide and lactosylceramide.

3. Use according to claim 1 or 2, wherein said specific catcher is an antibody or a lectin.

4. Use according to any one of the claims 1-3, wherein said pharmaceutical preparation is formulated for nasal, oral, subcutaneous, intramuscular, or intravenous administration.

5. Use according to any one of the claims 1-4, wherein said pharmaceutical preparation is intended for the prophylaxis of prediabetes, diabetes and/or associated complications in an individual being in risk of developing said disease, by inducing tolerance to the antigenic glycolipids.

6. Use according to claim 5, wherein said pharmaceutical preparation is intended for perinatal administration thereof to said individual.

7. Use according to any one of the claims 1-6, wherein said pharmaceutical preparation upon administration to an individual will lead to increased levels of suppressor or regulator cells or antibodies against lymphocytes recognising the antigenic glycolipids in said individual.

8. Use according to any one of the claims 1-6, wherein said pharmaceutical preparation upon administration to an individual will lead to the removal of antibodies and/or lymphocytes recognising the antigenic glycolipids from the blood stream of the individual.
9. Use according to any one of the claims 1-8, wherein said pharmaceutical preparation further comprises a least one bacterial adjuvant.

10. Use according to claim 9, wherein said bacterial adjuvant is cholera, staphylocococ or galactosylceramide (alpha-form) of bacterial origin.

11. Glycolipids, in particular galactosylceramide, glucosylceramide and lactosylceramide, and specific catchers therefore (antibodies or lectins) for use in the prophylaxis or therapy of prediabetes, diabetes and/or associated complications in an individual.

12. Galactosylceramide, glucosylceramide or lactosylceramide for use in the prophylaxis or therapy of prediabetes, diabetes and/or associated complications in an individual given nasally, orally, subcutaneously, intramuscularly, intravenously or otherwise.

13. Glycolipids according to claim 11 or 12, for use in the prophylaxis of prediabetes, diabetes and/or associated complications in an individual being in risk of developing said disease, by inducing tolerance to the antigenic glycolipids, for example by perinatal administration thereof to said individual.

14. Glycolipids according to claim 11 or 12, for use in the prophylaxis or therapy or prediabetes, diabetes and/or associated complications in an individual by raising suppressor or regulator cells or antibodies against lymphocytes recognising the antigenic glycolipids in said individual.

15. Glycolipids according to claim 11 or 12, for use in the prophylaxis or therapy of prediabetes, diabetes and/or associated complications in an individual by removing antibodies and/or lymphocytes recognising the antigenic glycolipids from the blood stream of the individual.

16. Glycolipids according to any one of the claims 11-15 given to an individual together with bacterial ad-
juvants, for example cholera, staphylococ or galactosylceramide (alpha-form) of bacterial origin.

17. A specific catcher for glycolipids according to any of claims 11-16, said catcher being a monoclonal antibody against galactosylceramide, glucosylceramide or lactosylceramide.

18. Antibody according to any of claims 11 and 17, for use in the prophylaxis or therapy of prediabetes, diabetes and/or associated complications in an individual by raising anti-antibodies in said individual.

19. A method for preventing the development of prediabetes, diabetes and/or associated complications in an individual, wherein a glycolipid, in particular galactosylceramide, glucosylceramide or lactosylceramide, is administered to said individual, preferably at its perinatal stage.

20. A method for preventing or treating prediabetes, diabetes and/or associated complications in an individual, wherein lymphocytes are removed from the individual, contacted with a glycolipid, in particular galactosylceramide, glucosylceramide or lactosylceramide, in vitro to make them recognise this antigen, irradiating the lymphocytes to inhibit their cytotoxicity, and (a) returning them to the individual to raise suppressor or regulator cells or antibodies against lymphocytes reactive with this antigen, or (b) administering them parenterally to another mammal in order to raise antibodies against lymphocytes reactive with this antigen in said mammal and then isolating serum containing the antibodies from said mammal and administering it to the individual.

21. A method of preventing or treating prediabetes, diabetes and/or associated complications in an individual, wherein the blood stream of the individual is contacted with an immobilised glycolipid, in particular galactosylceramide, glucosylceramide or lactosylceramide, to remove antibodies and/or lymphocytes recognising the antigenic glycolipids from the individual.
22. A method of preventing or treating prediabetes, diabetes and/or associated complications in an individual, wherein an antibody against glycolipids, in particular galactosylceramide, glucosylceramide or lactosylceramide, is parenterally administered (a) to said individual in a sufficient amount to raise anti-antibodies in said individual, or (b) to another mammal in order to raise anti-antibodies in said mammal and then isolating serum containing the anti-antibodies from said mammal and administering it to the individual.

23. A method according to any one of the claims 19-21, wherein the glycolipid is selected from the group consisting of galactosylceramide, glucosylceramide, or lactosylceramide.
The document is an International Search Report (ISR) for an international application. The IPC classification is A61K 31/70, A61K 39/00, C07H 15/04, C07H 15/10. The fields searched are A61K, C07H.

The report lists two cited documents:

- **WO 9219633 A1 (BUSHARD, KARSTEN), 12 November 1992 (12.11.92), See claims**: Relevant to claim No. 1-23.


- **WO 9742974 A1 (BUSHARD, KARSTEN), 20 November 1997 (20.11.97)**: Relevant to claim No. 1-23.

The date of the actual completion of the international search is 7 April 1999, and the date of mailing of the international search report is 20 April 1999.
INTERNATIONAL SEARCH REPORT

Box I  Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **X** Claims Nos.: 19-23
   because they relate to subject matter not required to be searched by this Authority, namely:
   Claims 19-23 relate to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/Rule 39.1(iv).
   Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

2. **☐** Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. **☐** Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II  Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. **☐** As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. **☐** As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. **☐** As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:  

4. **☐** No required additional search fees were timely paid by the applicant. Consequently, this international search report restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  

**Remark on Protest**  
**☐** The additional search fees were accompanied by the applicant’s protest.  
**☐** No protest accompanied the payment of additional search fees.

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