Title: ANTIBODY AGAINST AN EPITOPE OF THE B. BURGDORFERI FLAGELLAR BASAL ROD PROTEIN (fbrp)

B. burgdorferi fbrp epitope
Consensus
Human Il-1Ra

Abstract: An antibody specifically recognizing a peptide epitope of the Borrelia burgdorferi flagellar rod protein (fbrp) is provided. Said antibody can be used for the diagnosis of Multiple sclerosis or a predisposition thereof. Furthermore, an antibody directed against the fbrp epitope recognizing antibody (anti-idiotypic antibody) is provided. Said antibody is a suitable tool for the diagnosis and therapy of Multiple sclerosis.
Antibody against an epitope of the B. burgdorferi flagellar basal rod protein (fbbrp)

Technical Field

The present invention relates to an antibody against an epitope of the Borrelia burgdorferi flagellar rod protein (fbbrp) and the use of said antibody in diagnostic methods for Multiple sclerosis. Furthermore, the invention relates to an anti-idiotypic antibody directed to the fbbrp specific antibody and its diagnostic and therapeutical uses.

Background Art

Multiple sclerosis is a chronic, often disabling disease of the central nervous system. Symptoms may be mild such as numbness in the limbs or severe such as paralysis or loss of vision.

Most people with MS are diagnosed between the ages of 20 and 40 but the unpredictable physical and emotional effects can be lifelong.

Current methods of treatment include physical therapy, occupational therapy, speech therapy and medications such as β-interferon.

The causative agent of Lyme Disease is the bacteria Borrelia burgdorferi. Lyme disease which in its chronic form is thought to be an autoimmune disease mimics Multiple Sclerosis in several respects (Karussis et al., Mult Scler 1999, 6: 395-402).

Although there exist already diagnostic and therapeutical methods for Multiple sclerosis there is a need for improved diagnostic methods and therapies.
Disclosure of the Invention

Hence, it is a general object of the invention to provide an antibody specifically recognizing an epitope of the flagellar rode protein (fbpr) of B. burgdorferi wherein said epitope comprises the amino acid sequence set forth in Seq. Id. No. 1.

A second object of the invention relates to the use of said antibody in a diagnostic test for multiple sclerosis or a predisposition thereof.

A further object of the present invention is an anti-idiotypic antibody specifically recognizing the antibody directed to the fbpr peptide epitope of B. burgdorferi and its diagnostic and therapeutic uses.

Brief Description of the Drawing

The invention will be better understood and objects other than those set forth above will become apparent when consideration is given to the following detailed description thereof. Such description makes reference to the annexed drawing, wherein:

Figure 1 shows the molecular mimicry between B. burgdorferi fbpr and human interleukin-1 receptor antagonist (IL-1ra). Accession numbers [National center for biotechnology information (NCBI) protein database]: B. burgdorferi fbpr: 1448943; Human Interleukin-1 receptor antagonist: 999512.

Modes for Carrying Out the Invention

The inventor of the present invention has found that the Borrelia burgdorferi flagellar rod protein (fbpr) shares an epitope (antigenic determinant) with the human interleukin 1-receptor antagonist (IL-1ra) (see Figure 1). This phenomenon is termed molecular mimicry and explains why an antibody generated against a particu-
lar epitope of an infectious pathogen may become an auto-
antibody reacting with a homologous epitope in the host
and bringing about structural dysfunction or tissue dam-
age.

Interleukin 1 receptor antagonist is an anti-
inflammatory cytokine. Lower IL-1ra levels versus higher
interleukin 1 activity levels enhance inflammation in
Multiple sclerosis, experimental allergic encephalomyeli-
tis and Borrelia burgdorferi induced Lyme disease. Lyme
disease which in its chronic form is thought to be an
autoimmune disease mimics Multiple sclerosis in several
respects. Molecular mimicry of flagellar B. burgdorferi
epitopes might trigger and misdirect antibodies against
host tissue. It is therefore postulated that the flagel-
lar virulence factor fbrp which shares an epitope with
the human IL-1ra induces at birth antibodies against this
anti-inflammatory cytokine. Unleashing a harmful cascade
of pro-inflammatory cytokines, cognital infection during
parturition and re-exposure to the same or a similar an-
tigen would subsequently trigger Multiple sclerosis later
in life.

Based on these findings, the present inven-
tion provides new tools for the ex vivo diagnosis and
therapy of Multiple sclerosis.

The general object of the present invention
relates to an antibody specifically recognizing an epi-
tope of the flagellar rode protein (fbrp) of B. burgdor-
feri wherein said epitope comprises the amino acid se-
quence set forth in Seq. Id. No. 1. The peptide having
the amino acid sequence set forth in Seq. Id. No. 1 forms
the core of the epitope but said epitope can include fur-
ther flanking amino acids or amino acid insertions.

In a preferred embodiment said epitope com-
prises the amino acid sequence set forth in Seq. Id. No.
2 or Seq. Id. No. 3.

In another preferred embodiment said epitope
has the amino acid sequence set forth in Seq. Id. No. 1.
A further preferred embodiment relates to an antibody recognizing an epitope which has the amino acid sequence set forth in Seq. Id. No. 2 or Seq. Id. No. 3. The antibody of the present invention can be a polyclonal antibody as well as a monoclonal antibody. The antibody of the present invention is a suitable tool for the diagnosis of Multiple sclerosis or a predisposition thereof.

The man skilled in the art knows suitable methods for the production of polyclonal or monoclonal antibodies (see, for example, Antibodies: a laboratory manual / Ed Harlow, David Lane. Cold Spring Harbor, NY: Harbor Laboratory, 1988 and Harlow Edward. Using antibodies: a laboratory manual / Ed Harlow, David Lane. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1999.). A mammal, such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of a peptide epitope as defined in the present invention. Techniques for conferring immunogenicity on a peptide include conjugation to carriers or other techniques well known in the art. An epitope peptide of the present invention can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the epitope as antigen to assess the levels of antibodies.

Following immunization of an animal with an antigenic preparation of an epitope as defined in the present invention, anti-fbrp antisera can be obtained and, if desired, polyclonal anti-fbrp antibodies isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique (originally developed by (Kohler and
Milstein, Nature 256:495-7, 1975)), the human B cell hybridoma technique (Kozbar, Immunology Today 4:72, 1983), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, Monoclonal Antibodies and Cancer Therapy, 1985). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the epitope as defined herein and monoclonal antibodies isolated from a culture comprising such hybridoma cells.

The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with an epitope of the present invention. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(\(ab\))\(_2\) fragments can be generated by treating antibody with pepsin. The resulting F(\(ab\))\(_2\) fragment can be treated to reduce disulfide bridges to produce Fab fragments. The antibody of the present invention is further intended to include bispecific and chimeric molecules having affinity for an epitope of the present invention conferred by at least one CDR region of the antibody. The term antibody as used herein comprises as well single chain antibodies specific to an epitope as defined in the present invention. Methods for the production of such single chain antibodies is e.g. described in European Patent No. 0 281 604.

The above described antibodies of the present invention can be used in an ex vivo method for the diagnosis of Multiple sclerosis or a predisposition thereof.

The presence of the B. burgdorferi fbrp protein harboring the peptide epitope that is recognized by the antibodies of the first object of the present invention in an individual is associated with the disease Multiple sclerosis or a predisposition thereof. Therefore, the detection of an antigen that binds to the antibody of the present invention in an individual allows the diagno-
sis of Multiple sclerosis or allows a prediction whether said individual is at risk to be affected by Multiple sclerosis.

The detection of said antigen in an individual e.g. by detecting said antigen in a body liquid sample, tissue sample or blood sample of said individual, can be done by standard methods known in the art by means of the antibodies of the first object of the invention. The man skilled in the art knows suitable methods for the detection of antigens in e.g. a tissue and/or blood sample using antibodies. Said methods include but are not limited to Western blots, Enzyme Linked Immunosorbent Assay ("ELISA"), immunoprecipitations, slot or dot blots, radioimmunoassays, and fluorescent immunoassays.

A third object of the present invention is an antibody specifically binding to the antibody directed to a B. burgdorferi fbrp epitope as defined herein. Said antibody preferably binds the antigen combining site of the antibody directed to the B. burgdorferi fbrp epitope.

Such antibodies are referred to as anti-idiotypic antibodies. Most of these anti-idiotypic antibodies specifically recognize the antigen-combining sites of immunoglobulin molecules and therefore bind only immunoglobulin molecules with a particular antigenic specificity.


The anti-idiotypic antibody of the present invention can be used as tool in ex vivo diagnostic tests for the identification of Multiple sclerosis or a predisposition thereof.
An exemplary ex vivo diagnostic test for the detection of Multiple sclerosis comprises detecting in a body liquid sample or a tissue sample of an individual the presence of an antibody specifically recognizing the fbpr epitope of *B. burgdorferi* as defined herein using the anti-idiotypic antibody of the present invention.

The detection of said antibody in an individual e.g. by detecting said antibody in a body liquid sample, tissue sample or blood sample of said individual, can be done by standard methods know in the art by means of the anti-idiotypic antibodies of the present invention. The man skilled in the art knows suitable methods for the detection of proteins in e.g. a tissue and/or blood sample using antibodies. Said methods include but are not limited to Western blots, Enzyme Linked Immunosorbent Assay ("ELISA"), immunoprecipitations, slot or dot blots, radioimmunoassays, and fluorescent immunoassays.

The anti-idiotypic antibodies of the present invention can as well be used for the treatment of Multiple sclerosis by blocking the detrimental binding of the *B. burgdorferi* fbpr epitope recognizing antibody to tissue of an individual.

For therapeutic applications, the anti-idiotypic antibodies of the invention are administered to an individual, preferably a human, in a pharmaceutically acceptable dosage form. They are administered intravenously as a bolus or by continuous infusion over a period of time, by intramuscular, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

Such dosage forms encompass pharmaceutically acceptable carriers that are inherently nontoxic and non-therapeutic. Examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffers such as phosphate or glycine, sorbic acid, potassium sorbate,
partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes such as protamine sulfate, sodium chloride, metal salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulosic polymers, and polyethylene glycol. Conventional depot forms include, for example, microcapsules, nano-capsules, liposomes, plasters, sublingual tablets, and polymer matrices.

While there are shown and described presently preferred embodiments of the invention, it is to be distinctly understood that the invention is not limited thereto but may be otherwise variously embodied and practiced within the scope of the following claims.
Claims

1. An antibody specifically recognizing an epitope of the flagellar rode protein (fbpr) of B. burgdorferi wherein said epitope comprises the amino acid sequence set forth in Seq. Id. No. 1.

2. The antibody of claim 1 wherein said epitope comprises the amino acid sequence set forth in Seq. Id. No. 2 or Seq. Id. No. 3.

3. The antibody of claim 1 wherein said epitope has the amino acid sequence set forth in Seq. Id. No. 1.

4. The antibody of claim 2 wherein said epitope has the amino acid sequence set forth in Seq. Id. No. 2 or Seq. Id. No. 3.

5. The antibody according to any one of claims 1-4, wherein said antibody is a polyclonal antibody.

6. The antibody according to any one of claims 1 to 4, wherein said antibody is a monoclonal antibody.

7. Use of the antibody according to any one of the preceding claims in an ex vivo diagnostic method for the detection of Multiple sclerosis or a predisposition thereof.

8. An anti-idiotypic antibody specifically recognizing an antibody according to any one of claims 1-6.
9. Use of the anti-idiotypic antibody according to claim 8 as tool for the ex vivo diagnosis of Multiple sclerosis or a predisposition thereof.

10. Use of the anti-idiotypic antibody according to claim 8 for the therapy of Multiple sclerosis.

11. The anti-idiotypic antibody of claim 8 as pharmaceutical.

12. Use of the anti-idiotypic antibody of claim 8 for the manufacturing of a medicament for the treatment of Multiple Sclerosis.

13. A pharmaceutical composition for the treatment of Multiple Sclerosis comprising the anti-idiotypic antibody of claim 8.
Fig.1

*B. burgdorferi fbrp epitope* 106 **GYVELPNVNLVEEMVDMI** 123
Consensus

Human IL-1Ra 34 **GYLQGPNVNL EEKIDVV** 50
SEQUENCE LISTING

Fritzsche, Markus

Antibody against an epitope of the B. burgdorferi flagellar basal rod protein (fbpr)

06325PC

3

PatentIn version 3.1

1

10

PRT

Borrelia burgdorferi

1

Gly Tyr Pro Asn Val Asn Leu Glu Glu Asp

1 5 10

2

18

PRT

Borrelia burgdorferi

2

Gly Tyr Val Glu Leu Pro Asn Val Asn Leu Val Glu Glu Met Val Asp

1 5 10 15

Met Ile

3

17

PRT

Homo sapiens

3

Gly Tyr Leu Gln Gly Pro Asn Val Asn Leu Glu Glu Lys Ile Asp Val

1 5 10 15

Val
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/12 G01N33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, BIOTECHNOLOGY ABS, CHEM ABS Data, EMBASE, LIFESCIENCES, MEDLINE, PAJ, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>X</td>
<td>WO 01 89549 A (FORD JOHN ; HYSEQ INC (US) ; H0 ALICE (US)) 29 November 2001 (2001-11-29) page 20, line 12 -page 21, line 17; claims 1,5,7,8,21,23 --- ---</td>
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Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :
  **A** document defining the general state of the art which is not considered to be of particular relevance.
  **E** earlier document but published on or after the international filing date.
  **L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified).
  **O** document referring to an oral disclosure, use, exhibition or other means.
  **P** document published prior to the international filing date but later than the priority date claimed.
  **Y** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention.
  **X** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone.
  **Y** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
  **K** document member of the same patent family.

Date of the actual completion of the international search 28 June 2002

Date of mailing of the international search report 12/07/2002

Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax. (+31-70) 340-3016

Authorized officer Pilat, D

Form PCT/ISA/210 (second sheet) (July 1992)
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<td>WO 00 64479 A (IGNATYEV GEORGE M; FREDEKING TERRY M (US); ANTIBODY SYSTEMS INC (U) 2 November 2000 (2000-11-02) example 12</td>
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<td>US 6 183 755 B1 (PREAC-MURSIC VERA ET AL) 6 February 2001 (2001-02-06) abstract column 3, line 39 - line 45; example 6; table 2</td>
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<td>WO 01 58956 A (BASF AG; BROCKLEHURST SIMON MARK (GB); DUNCAN ALEXANDER ROBERT (GB) 16 August 2001 (2001-08-16) claims 1,56,59; figure 1; tables 1,5</td>
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Continuation of Box I.2

Claims Nos.: 8-13

Present claims 8-13 relate to a product and use of said product defined by reference to a desirable characteristic or property, namely being an anti-idiotypic antibody of those specifically recognizing an epitope comprising SEQ ID NO1-3.

The claims cover all products having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for such products.

In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely on the process for obtaining said compounds as mentioned in the description at page 6 lines 26-33 and on the concept of using such anti-idiotypic antibodies.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.
### 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.☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2.☒ Claims Nos.: 8-13
   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:
   see FURTHER INFORMATION sheet PCT/ISA/210

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 is 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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