(54) Title: COMPOSITION FOR PROPHYLAXIS AND TREATMENT OF PULMONARY FIBROSIS

(57) Abstract

A monoclonal antibody to either or both of the leukocytic α sub-units CD-11a and CD-11b is used for the manufacture of a composition for the prophylaxis and treatment of pulmonary fibrosis in mammals. A method of prophylaxis and treatment of pulmonary fibrosis in mammals by the administration of an effective amount of a monoclonal antibody to either or both of the leukocytic α sub-units CD-11a and CD-11b is also provided.
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COMPOSITION FOR PROPHYLAXIS AND TREATMENT OF
PULMONARY FIBROSIS

The invention relates to a method of prophylaxis and treatment of pulmonary fibrosis. Furthermore, it relates to a pharmaceutical composition for the prophylaxis and treatment of pulmonary fibrosis. More generally it relates to the facilitation of wound repair.

Pulmonary fibrosis is any one of a group of diseases characterised by an increase in the deposition of proteins of the extra-cellular matrix (ECM), notably collagens, generally associated with the growth of interstitial cells. These diseases respond poorly to current therapy, and are responsible for about 5% of annual deaths in the USA (see Bitterman and Henke in Chest, 1991, 99, 81s). The onset of pulmonary fibrosis is frequently associated with tissue injury which results in parenchymal death, leading to a complex pattern of responses being engaged which should lead to organ repair. However, under certain circumstances, instead of repair being initiated, a fibroproliferative response may ensue, in which parenchyma is replaced by mesenchymal cells, new capillaries, and their connective tissue products. The reason why injury sometimes leads to complete repair, while other times it results in fibrosis is as yet poorly understood.
Several fibrogenic cytokines have been characterized recently which might be involved in pulmonary fibrosis, notably tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), transforming growth factor (TGF-β) or platelet derived growth factor (PDGF) (see E. J. Kovacs, Immunology Today, 1991, 12, 17). Macrophages are known to produce several of these factors. The lung contains various leukocytes (i.e. lymphocytes, macrophages and polynuclear leukocytes), in three distinct compartments – alveolar, interstitial and intra-vascular (see Abraham et al, J. Immunol., 1990, 144, 2117), which may also play some role in the development of pulmonary fibrosis instead of normal healing of lesions.

The two most commonly used experimental models in the study of pulmonary fibrosis are the pulmonary fibrosis elicited by the instillation of either bleomycin or silica (see D. H. Bowden, Laboratory Investigation, 1984, 50, 487, and E. M. Lugano et al, Am. J. Pathol., 1982, 109, 27). It has been shown that development of bleomycin-induced pulmonary fibrosis can be prevented by a depletion of T lymphocytes (Piguet et al, J. Exp. Med., 1989, 170, 655), the presence of T-lymphocytes being necessary for both increased lung TNF mRNA levels and the development of fibrosis. Silicosis, however, the other commonly used model for
pulmonary fibrosis, has been shown not to be markedly affected by the absence of T-lymphocytes. It has also been shown that administration of anti-TNF antibodies to mice during instillation of silica almost completely prevents deposition of collagen due to silica-induced pulmonary fibrosis (P.F. Piquet et al, Nature, 1990, 344, 245). Administration of anti-TNF antibodies, however, is much less effective in the prevention of pulmonary fibrosis induced by bleomycin (P.F. Piquet et al, J. Exp. Med., 1989, 170, 655). Thus, although much research interest has centered on the possible development of the work with T-lymphocytes and anti-TNF antibodies, it has become clear that neither approach is likely to provide a viable method of treatment of the complete range of pulmonary fibrosis.

It is the aim of the present invention to provide a method of prophylaxis and treatment of pulmonary fibrosis. A pharmaceutical composition is sought that may be administered both as a means of preventing the onset of pulmonary fibrosis, and treating the condition after its onset.

As mentioned earlier, it is possible that the leukocytes of the lung may have some role to play in the evolution of pulmonary fibrosis. The interactions and state of activation of leukocytes is known to be modulated by various surface proteins. One such class
of surface proteins is the leukocytic integrins. There are three leukocytic integrins, all of which are non-covalently linked heterodimers sharing a common $\beta$-subunit (CD-18) and different but homologous $\alpha$-subunits: the CD-11a (or the LFA-1), the CD-11b (or Mac-1 or CR3), and the CD-11c (or the p150, 95) (see Anderson and Springer in Ann. Rev. Med., 1987, 38, 175).

The CD-11/CD-18 family, also known as the leukocytic or $\beta$-2 integrins, are believed to be involved in various immune and inflammatory responses on the basis of two pieces of evidence. Firstly, CD-18 congenital deficiencies in humans lead to a severe disease, known as Leukocyte Adhesion Deficiency (LAD), which is characterised by recurrent bacterial infections, and impaired pus formation and wound healing (see Anderson and Springer in Ann. Rev. Med., 1987, 38, 175). LAD can be treated by bone marrow transplantation, donor rejection of HLA-mismatched bone marrow being overcome by administration of anti CD-11a mAbs to the patient from about 3 to 5 days before the graft. Secondly, the administration of anti CD-11 or anti CD-18 mAb to rodents affects the course of several immune related disorders e.g. the late administration of monoclonal antibody to LFA-1 (CD-11a) has been found to abrogate incipient murine cerebral malaria (see Grau et al, Eur. J. Immunology, 1991, 21, 2265).
In the course of studies on pulmonary fibrosis, we have made the surprising discovery that pulmonary fibrosis elicited by the intratracheal instillation of either bleomycin or silica (the two commonly used pulmonary fibrosis models, as described above), can be completely prevented by the administration of anti CD-11a or anti CD-11b mAb during the period over which it is elicited. Furthermore, administration of anti CD-11a or anti CD-11b mAb has also been found to be extremely effective even after the establishment of a pulmonary fibrosis, further collagen deposition being completely prevented.

In the present invention, therefore, we provide a method of prophylaxis and treatment of pulmonary fibrosis in mammals by the administration of an effective amount of a monoclonal antibody to either or both of the leukocytic α sub-units CD-11a and CD-11b.

Normally, as experimental models, pulmonary fibrosis may be elicited by the intratracheal instillation of bleomycin or silica in mice, significant increases in the lung hydroxyproline content being observed after 15 days. However, simultaneous administration of either or both of anti CD-11a or anti CD-11b mAb during the period of instillation has been found to prevent excessive lung collagen deposition for both bleomycin and silica.
installation, i.e. administration of the antibodies completely prevents the onset of both bleomycin and silica induced pulmonary fibrosis. Indeed, collagen levels in the lung, as measured by the lung hydroxyproline content, are slightly lower after administration of anti CD-11 mAbs following the installation of silica or bleomycin, than those in normal lungs in which no fibrosis have been elicited.

As has been discussed earlier, previous attempts at preventing pulmonary fibrosis (e.g. depletion of the T-lymphocyte population), have been at least partly successful in preventing the onset of one of the experimental pulmonary fibrosis, but never both. The extraordinary success of the administration of anti CD-11a and/or anti CD-11b mAbs in preventing the onset of both types of experimental fibrosis is consequently a highly significant breakthrough. It is clear from these studies that administration of either or both of the anti CD-11 mAbs represents a significant method of prophylaxis of pulmonary fibrosis.

Furthermore, we have also found that administration of either or both of the anti CD-11a and anti CD-11b mAbs, after pulmonary fibrosis has already been established in mice by the administration of bleomycin or silica, is effective in completely reversing the advance of the fibrosis, no further collagen deposition
being observed. This is extremely surprising, since none of the previous treatments for pulmonary fibrosis shows anything approaching such a complete reversal of both bleomycin and silica induced fibrosis.

Administration of the mAbs during either prophylaxis or treatment was found to have little effect on the number of alveolar leukocytes (mainly composed of macrophages). The administration of the anti CD-11 mAbs did appear, however, to affect the function or number of the lung leukocytes. The evaluation of the lung TNF mRNA levels is in agreement with these findings: both silica and bleomycin induced fibrosis are associated with a marked rise in the TNF mRNA levels, but the TNF mRNA levels were completely unaffected by administration of either anti CD-11a or anti CD-11b mAbs, which is as would be expected if there is no change in the macrophage population. Similarly, the mRNA levels of other fibrogenic cytokines, such as IL-1, TGF-β and PDGF, were not significantly decreased in anti CD-11 mAbs treated mice.

It is believed that the administration of anti CD-11 mAbs to humans may be of considerable benefit in the prophylaxis and treatment of a wide range of pulmonary fibrosis including idiopathic pulmonary fibrosis, drug-induced pulmonary fibrosis, silicosis, pneumoconiosis (e.g. asbestosis, berylliosis), and acute
respiratory distress syndrome (ARDS).

In a further aspect of the present invention, we provide use of a monoclonal antibody to either or both of the leukocytic α sub-units CD-11a and CD-11b for the manufacture of a composition for the prophylaxis and treatment of pulmonary fibrosis in mammals.

The invention may be further illustrated by consideration of the Examples below. The animals, materials and methods used in the Examples are as follows:

Mice

CBA/Ca and C57BL/10 (B10) mice were purchased from OLAC Ltd. (Blackthorn, UK), and bred for 3-4 generations. Experiments were performed with 2-4 month old male mice.

Bleomycin and Silica Administration

Bleomycin (Lundbeck, AVA, Copenhagen, Denmark) was dissolved in HBSS and 0.08 U in 0.1 ml was injected intra-tracheally. This procedure induced some morbidity, manifested by a loss in body weight and death by the 15th day after the injection in 5-20% of the mice. About 2 mg silica particles (DQ 12, size < 5 micron) were suspended in 0.1 ml of saline, and injected
intra-tracheally. This treatment did not induce detectable loss of body weight or mortality.

**Anti CD-11 mAbs**

Anti CD-11a - rat anti LFA-1 IgG2b (H35.89.9), isolated as per Grau et al, Eur. J. Immunol., 1991, 21, 2265, was used.

Anti CD-11b - rat anti CR3 IgG2b (5C6), isolated as per Rosen et al, J. Exp. Med., 1987, 166, 1685, was used.

**Lung Hydroxyproline Content**

This was established according to the procedure of Thrall et al, Am. J. Pathol., 1979, 95, 117. In brief, the lungs were submitted to an acid hydrolysis and the hydrolysates were neutralised and extracted with phenol-chloroform-isoamyl alcohol to clarify the aqueous phase. The hydroxyproline concentration was then determined colorimetrically (J.F. Woessner, Arch. Biochem. Biophys., 1961, 93, 440).

**Lung Alveolar and Interstitial Cells**

The alveolae were lavaged with saline instilled within the trachea by a 25 cm hydrostatic pressure. Lung interstitial cells were isolated as described by Abraham

Microscopy

The lungs were fixed by the intra-tracheal administration of formol sublimate or glutaraldehyde (2% in 0.1M cacodylate buffer, pH 7.4) for paraffin or methacrylate and epon embedding respectively. Four sections from paraffin embedded material for each individual mouse were stained with hematoxilin and eosin, and a modified trichrome stain was used for the identification of platelet aggregates.

Northern Blot Analysis of TNF-a and IL-1-a mRNA

The lungs were washed in saline and frozen in liquid nitrogen. They were subsequently thawed and minced in guanidine-thiocyanate solution and the total lung RNA was isolated by guanidine/caesium chloride centrifugation. RNAs, denatured with glyoxal, were separated on 1.2% agarose gels (4 μg per lane), and transferred onto nylon membranes. Filters were hybridised with $^{32}$P-labelled cRNA probes (see Collart et al, J. Exp. Med., 1986, 163, 2113).
Example 1

Prevention of Bleomycin or Silica Induced Collagen Deposition by CD-11 mAbs

Pulmonary fibrosis were elicited in groups of mice by the instillation of bleomycin or silica as described above. 500 μg of the anti CD-11a or anti CD-11b mAbs were injected every 5 days after instillation, and the mice were sacrificed on the 15th day after instillation. Control tests were run in which saline was injected intra-tracheally at the beginning of the experiment, and in which saline was injected into the mice at 5 day intervals instead of the antibodies after instillation by bleomycin or silica. Cells from the broncho-alveolar lavage (BAL) were recovered after an intra-tracheal instillation of saline. The lung hydroxyproline, as an evaluation of the lung collagen, was determined after lung hydrolysis. The results were as shown in Table 1 below.
### Table I

**Prevention of bleomycin or silica induced collagen deposition by CD-11 mAbs**

<table>
<thead>
<tr>
<th>Instillation</th>
<th>Treatment</th>
<th>BAL ( \times 10^{-4} )</th>
<th>Lung h-proline (µg/lung)</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>none</td>
<td>1.5 (1)</td>
<td>97 (7)</td>
</tr>
<tr>
<td>silica</td>
<td>saline</td>
<td>18 (4)</td>
<td>144 (48)</td>
</tr>
<tr>
<td>silica</td>
<td>anti CD-lla</td>
<td>10 (4)</td>
<td>89 (30)*</td>
</tr>
<tr>
<td>silica</td>
<td>anti CD-llb</td>
<td>16 (3)</td>
<td>80 (39)*</td>
</tr>
<tr>
<td>bleomycin</td>
<td>saline</td>
<td>12 (3)</td>
<td>131 (18)</td>
</tr>
<tr>
<td>bleomycin</td>
<td>anti CD-lla</td>
<td>13 (4)</td>
<td>88 (27)**</td>
</tr>
<tr>
<td>bleomycin</td>
<td>anti CD-llb</td>
<td>11 (3)</td>
<td>87 (16)**</td>
</tr>
</tbody>
</table>

Results are the mean (+sd) of the values obtained with 7-10 mice. The significance of the difference with the saline treated group was evaluated by the Mann and Whitney non-parametric U test; *p < 0.02, **p < 10^{-2}.

From these results it can be seen that in the control experiment, where saline only is instilled at the start of the test, the mean hydroxyproline content per lung was 97µg/lung. When only saline is administered to
the mice after instillation of silica or bleomycin, there is a dramatic increase in the lung hydroxyproline content by the 15th day in both cases, when compared to the control. When either anti CD-11a or anti CD-11b mAbs are administered over the 15 day period after instillation with silica or bleomycin, however, the lung hydroxyproline content is actually slightly lower than that in the control mice.

Clearly, these results are highly significant in terms of providing a method of prophylaxis for pulmonary fibrosis, given the remarkable degree to which collagen deposition in the lungs is prevented by administration of anti CD-11a or anti CD-11b mAbs.

Example 2

Pulmonary fibrosis was elicited in mice using bleomycin or silica as in Example 1. Anti CD-11a mAbs (500 μg) were administered to the mice on day 20 and day 25 and the mice were sacrificed on day 30 after silica or bleomycin instillation. Control tests were run in which saline was injected at days 20 and 25 after instillation with silica or bleomycin instead of the mAbs. Mice, to which nothing had been administered throughout the period of the tests, were also sacrificed after 30 days. The results were as shown below in Table II.
### Table II

**Treatment of an established fibrosis with anti CD-11 mAb**

<table>
<thead>
<tr>
<th>Instillation</th>
<th>Treatment</th>
<th>Lung h-proline μg/lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>none</td>
<td>103 (7)</td>
</tr>
<tr>
<td>silica</td>
<td>saline</td>
<td>120 (22)</td>
</tr>
<tr>
<td>silica</td>
<td>anti CD-11a</td>
<td>63 (10)*</td>
</tr>
<tr>
<td>bleomycin</td>
<td>saline</td>
<td>151 (37)</td>
</tr>
<tr>
<td>bleomycin</td>
<td>anti CD-11a</td>
<td>106 (10)**</td>
</tr>
</tbody>
</table>

Results are the mean (+sd) of groups of 5 mice.
Difference with the saline injected group; *p < 10^{-3},
**p < 10^{-2}.

When only saline is injected after instillation of silica or bleomycin, there is a significant increase in lung hydroxyproline content by day 30, when compared to the control mice where no silica or bleomycin had been instilled, as would be expected. When anti CD-11a mAbs are administered after days 20 and 25 (i.e. after the pulmonary fibrosis have been established), it is found
that in the case of bleomycin induced fibrosis, the lung hydroxyproline content is approximately the same as the value for mice in which no fibrosis have been elicited, while in the case of silica induced fibrosis, the lung hydroxyproline content is actually below that of the value obtained for mice in which no fibrosis have been elicited.

Hence, from the above it is clear that not only does the administration of anti CD 11 mAbs prevent the onset of pulmonary fibrosis if administered immediately after lung injury, it also provides a highly effective form of treatment of pulmonary fibrosis once it has already been established, effectively reversing the condition.

**Example 3**

Pulmonary fibrosis was elicited using silica or bleomycin in groups of 4-8 mice as described in Example 1. On day 15 the lungs were fixed by an intra-tracheal instillation of formol sublimate, and 3-4 sections across the hilus of the major lobes were prepared for each individual mouse. Sections were scored semi-quantitatively as follows:

0 = normal; 1 = about 10% of parenchyma altered; 2 = about 25%; and 3 = about 50%.
Platelet microthrombi were scored per lung section, using a modified trichrome stain (see K.C. Carstairs, J. Path. Bact., 1965, 90, 225).

Lymphoid infiltration was evaluated as the number of lymphoid foci (i.e. lymphoid formation of $>20$) per section.

3-4 sections from 4-8 individual mice for each group were examined. Results are a mean (+sd) of the score obtained for each individual mouse.

The results were as shown in table III below.

**Table III**

<table>
<thead>
<tr>
<th>Instillation</th>
<th>Treatment</th>
<th>Histological score</th>
<th>Platelet thrombi</th>
<th>Lymphoid infiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica</td>
<td>solvent</td>
<td>0.9 (0.6)</td>
<td>1.2 (1.0)</td>
<td>2.2 (1.4)</td>
</tr>
<tr>
<td>Silica</td>
<td>(A)</td>
<td>0.7 (0.5)</td>
<td>0.1 (0.1)</td>
<td>1.2 (1.1)</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>solvent</td>
<td>1.3 (0.5)</td>
<td>1.7 (1.3)</td>
<td>3.6 (2.4)</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>(A)</td>
<td>1.2 (0.4)</td>
<td>0.0</td>
<td>0.5 (0.6)</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>(B)</td>
<td>0.9 (0.5)</td>
<td>0.0</td>
<td>1.1 (0.7)</td>
</tr>
<tr>
<td>none</td>
<td>solvent</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>none</td>
<td>(A)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
CLAIMS

1. Use of a monoclonal antibody to either or both of the leukocytic α sub-units CD-11a and CD-11b for the manufacture of a composition for the improvement in wound repair mechanisms in mammals.

2. Use of a monoclonal antibody to either or both of the leukocytic α sub-units CD-11a and CD-11b for the manufacture of a composition for the prophylaxis and treatment of pulmonary fibrosis in mammals.

3. Use according to Claim 2, in which a monoclonal antibody to the leukocytic α sub-unit CD-11a is used for the manufacture of a composition for the treatment of pulmonary fibrosis in mammals.
**INTERNATIONAL SEARCH REPORT**

**INTERNATIONAL APPLICATION NO.**
PCT/GB 93/00886

**I. CLASSIFICATION OF SUBJECT MATTER**
According to International Patent Classification (IPC) or to both National Classification and IPC

| Int.Cl. 5 | A61K39/395 |

**II. FIELDS SEARCHED**
Minimum Documentation Searched

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Documentation Searched other than Minimum Documentation

to the extent that such documents are included in the fields searched

**III. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No.</th>
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<tr>
<td>X</td>
<td>BIORHEOLOGY vol. 27, no. 3-4, 1990, OXFORD, GB pages 425-432 J. WAUTIER ET AL. 'Leukocyte adhesion to endothelial cells.' see the whole document ---</td>
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<td>P,X</td>
<td>AMERICAN REVIEW OF RESPIRATORY DISEASE vol. 145, no. 4(2), April 1992, NEW YORK, USA page A190 P. PIGUET ET AL. 'Antibody to the leukocyte integrins CD11a or b prevent or cure pulmonary fibrosis elicited in mice by bleomycin or silica.' see upper-left abstract ---</td>
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**IV. CERTIFICATION**

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<th>Date of Mailing of this International Search Report</th>
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<td>23-09-1993</td>
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International Searching Authority
EUROPEAN PATENT OFFICE

Signature of Authorized Officer
NOOIJ F. J. M.
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<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No.</th>
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| P,X      | AMERICAN REVIEW OF RESPIRATORY DISEASE  
v. 147, no. 2, February 1993, NEW YORK, USA  
pages 435 - 441  
P. PIGUET ET AL. 'Effective treatment of  
the pulmonary fibrosis elicited in mice by  
bleomycin or silica with anti-CD11  
antibodies.'  
see the whole document | 1-3 |