



No. 08-105676-00003-0000

Fecha: 2010-05-25 14:34:07 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 400 OPOSIOBSERVTER Folios: 66

FORMULARIO ÚNICO DE OPOSICIÓN

①

OPOSICIÓN A:

- | | |
|--|--|
| ☒ Patente de Invención. | ☐ Marca Colectiva. |
| ☐ Patente de Modelo de Utilidad. | ☐ Marca de Certificación. |
| ☐ Diseño Industrial. | ☐ Lema Comercial. |
| ☐ Esquema de Trazado para Circuitos Integrados. | |
| ☐ Marcas de Productos o Servicios. | |

②

Número del expediente: 8-105676

Solicitante: ELAN PHARMACEUTICALS, INC.

**SOLICITUD
PUBLICADA**

Apoderado: EMILIO FERRERO

Título de la nueva creación o denominación del signo: METODO PARA TRATAR
ENFERMEDADES INFLAMATORIAS Y AUTOINMUNES CON NATALIZUMAB

Gaceta: 613

③ **OPOSICIÓN PRESENTADA POR:**

SOLICITANTE		IDENTIFICACIÓN
Nombre: <u>PROCAPS S.A.</u>		C.C. ☐ NIT ☒
Dirección: <u>CALLE 80 No. 78b-201</u>		C.E. ☐ Otro ☐
Nacionalidad o Domicilio: <u>COLOMBIA</u>		Cual <u> </u>
Lugar de Constitución: <u>BARRANQUILLA</u>		Número <u>890.106.527-5</u>
Teléfono: <u>371 99 00</u> Fax: <u>373 08 18</u>		
E-mail: <u> </u>		
REPRESENTANTE O APODERADO		IDENTIFICACIÓN
Nombre: <u>CIELO ANGELA PEÑA RODRIGUEZ</u>		C.C. ☒ NIT ☐
Dirección: <u>CARRERA 13 No. 119-95</u>		C.E. ☐ Otro ☐
Teléfono: <u>(571)213 12 13</u> Fax: <u>(571)612 19 79</u>		Cual <u> </u>
E-mail: <u>negocios@optimum-co.com</u>		Número <u>51.940.252</u> TP <u>82701</u>

④ **FUNDAMENTOS DE LA OPOSICIÓN:**

Art.14, 16, 18, 20 y 30 de la Decisión 486 de la CAN

Causal: No cumple con los requisitos de patentabilidad.

Signo opositor:

Justificación:

La presente solicitud no cumple con los requisitos de patentabilidad dado que lo
revelado, ya se encontraba en el estado de la técnica o es posible derivarlo de la
misma tal y como se demostrará en el capítulo "Fundamento de Hechos".

⑤ Comprobante de pago No. _____

Fecha _____

⑥ ANEXOS

- ☒ Comprobante de pago de la tasa.
- ☒ Poderes, si fuere el caso.
- ☒ Pruebas de los fundamentos de la oposición.
- ☐ Documentos que acrediten el legítimo interés, si fuere el caso.
- ☒ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas.

⑦

NOMBRE: CIELO ANGELA PEÑA RODRIGUEZ

FIRMA: 

C.C. 51940252

TP. 82701



Derecho de la Competencia, Propiedad
Industrial y Comercio Exterior

Señora Jefe,
DIVISIÓN DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Referencia : Expediente N° 08 105676 ✓
Asunto : Oposición a la solicitud de patente de Invención titulada
"METODO PARA TRATAR ENFERMEDADES
INFLAMATORIAS Y AUTOINMUNES CON
NATALIZUMAB".
Solicitante : ELAN PHARMACEUTICALS, INC.
Opositora : PROCAPS S.A.

Publicada en la Gaceta de la Propiedad Industrial ✓
N° 613 del 19 de febrero de 2010

Yo, *CIELO ANGELA PEÑA RODRIGUEZ*, abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. *82701* del Consejo Superior de la Judicatura, en mi condición de apoderada de la sociedad **PROCAPS S.A.** conformada bajo las leyes de la República de Colombia y domiciliada en la ciudad de Barranquilla, Colombia, atentamente manifiesto que estando dentro del término de ley y por orden expresa de mi representada, presento oposición a la concesión del registro de la Patente de Invención titulada "**METODO PARA TRATAR ENFERMEDADES INFLAMATORIAS Y AUTOINMUNES CON NATALIZUMAB**", cuyo titular es **ELAN PHARMACEUTICALS, INC.**, y pido a Usted declarar fundada esta oposición y en consecuencia negar el registro de la patente solicitada.

1. INTERÉS PARA ACTUAR

Conforme a lo dispuesto en el artículo 42 de la Decisión 486 de 2000 de la CAN, estando dentro del término estipulado por la ley, manifiesto el legítimo interés de nuestra representada para oponerse al registro de la patente de la referencia, atendiendo a que la solicitud pretendida no cumple con los requisitos dispuestos en la ley como se analizará más adelante y de ser concedida afectaría directamente la comercialización de algunos productos de mi representada, que contienen, en particular NATALIZUMAB. Por lo tanto, solicito respetuosamente a ese Despacho se sirva tener en cuenta la presente oposición dentro del trámite administrativo que se surte en esta Superintendencia.

2. FUNDAMENTOS DE HECHOS

2.1. La solicitud objeto de la presente fue presentada el 03 de octubre de 2008 reivindicando prioridad bajo la solicitud US20060779190P del 20060303; y fue publicada en Colombia, en la Gaceta de la Propiedad Industrial N° 613 del 19 de febrero de 2010, cuya publicación internacional corresponde a la WO2007/103112 del 2007-09-13.

La solicitud colombiana 08 105676 objeto de la presente oposición, se refiere a un método para tratar un paciente con una enfermedad inflamatoria o auto-inmune con natalizumab que comprende: (a) administrar una dosis de natalizumab durante un



primer periodo de dosis; (b) monitorear la cantidad de natalizumab bivalente en el plasma o suero del paciente durante el primer periodo de dosis; (c) determinar una segunda dosis de natalizumab con base en el nivel de natalizumab bivalente observado; y (d) administrar una segunda dosis de natalizumab durante un segundo periodo de dosis; en donde la segunda dosis mejora la seguridad y/o eficacia del tratamiento durante el segundo periodo de dosis.

2.2. En primera instancia, se debe advertir que en el capítulo reivindicatorio de la solicitud objeto de la presente oposición, se hace referencia a un método terapéutico, no sólo a la composición farmacológica o a un método de fabricación mediante la cual se obtenga un producto tangible y según la legislación, decisión 486 de la Comunidad Andina de Naciones, Artículo 14.- *“Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento (...)”*, los usos no están contemplados como patentables; y, Artículo 20.- *“No serán patentables (...) d) los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales”*. Solo serán patentables los productos o procedimientos. Por lo tanto a luz de la norma, dicha materia referente a métodos terapéuticos no sería patentable.

2.3. Con base en el artículo 14 de la Decisión 486 de la CAN que consagra: *“Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial”*, tenemos que las invenciones objeto de patente deben ser nuevas y tener nivel inventivo. Bien, pues antes de la fecha de prioridad de esta solicitud se encuentran los siguientes documentos que afectan la patentabilidad de la presente solicitud:

- D1: US2005065158 publicada el 24 de marzo de 2005.

- D2: "Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites. Immunologia, volumen 97 páginas 693 a 698 publicado en 1999.

2.4. Ahora bien, el documento D1, particularmente en la descripción se refiere a Un método para reducir crónicamente la inflamación patológica de un paciente vía la administración de un agente que expresamente se une a una integrina alfa 4 o un dímero que comprende una integrina alfa 4. El agente debe tener una afinidad obligatoria tal que la administración es suficiente para suprimir la inflamación patológica, y el agente es administrado crónicamente para proporcionar la supresión a largo plazo de inflamación patológica. El documento D2 más adelante en la descripción párrafo 013 refiere el natalizumab como necesario para el método presentado y adicionalmente el mismo documento D1 en el párrafo 176 revela el monitoreo de la cantidad de natalizumab en el plasma o suero de los pacientes.

2.5. Por su parte, el documento D2 revela que un anticuerpo igG4 (como el natalizumab) se somete a una transformación en donde resulta un anticuerpo igG4 biespecífico y funcionalmente monovalente.

2.6. Tomando los documentos D1 y D2 como el estado del arte más cercano a la solicitud objeto de la presente oposición, no es posible reconocer altura inventiva, ya que sería obvio para un experto en la materia a partir de dicho documento D1, combinando los métodos con D2, concebir un método para tratar un paciente con una enfermedad inflamatoria o auto-inmune con natalizumab que comprende: (a) administrar una dosis de natalizumab durante un primer periodo de dosis; (b) monitorear la cantidad de natalizumab bivalente en el plasma o suero del paciente durante el primer periodo de dosis; (c) determinar una segunda dosis de

natalizumab con base en el nivel de natalizumab bivalente observado; y (d) administrar una segunda dosis de natalizumab durante un segundo periodo de dosis; en donde la segunda dosis mejora la seguridad y/o eficacia del tratamiento durante el segundo periodo de dosis.. Así entonces las reivindicaciones de la solicitud objeto de la presente oposición, carecen de altura inventiva.

2.7. En consecuencia y de acuerdo a lo descrito anteriormente, se deriva que la solicitud colombiana 08 105676 no puede pretender un monopolio por patente toda vez que lo que pretende no cumple con uno de los tres requisitos necesarios para su protección.

2.8. Que por consiguiente, respecto a la solicitud pretendida se tiene que:

2.8.1. CARENIA DE ALTURA INVENTIVA. Según la Decisión 486 de la Comunidad Andina de Naciones, Artículo 18.- *“Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica.”*. A la fecha de prioridad, 93 de marzo de 2006, a partir del documento D1 por si solo o en combinación con D2, puede llegar de manera evidente a un método para tratar un paciente con una enfermedad inflamatoria o auto-inmune con natalizumab que comprende: (a) administrar una dosis de natalizumab durante un primer periodo de dosis; (b) monitorear la cantidad de natalizumab bivalente en el plasma o suero del paciente durante el primer periodo de dosis; (c) determinar una segunda dosis de natalizumab con base en el nivel de natalizumab bivalente observado; y (d) administrar una segunda dosis de natalizumab durante un segundo periodo de dosis; en donde la segunda dosis mejora la seguridad y/o eficacia del

tratamiento durante el segundo periodo de dosis., sobre lo cual busca protección en el capítulo reivindicatorio.

2.8.2. CARENCIA DE APLICACIÓN INDUSTRIAL La Decisión 486 de la CAN consagra en su artículo 14 lo siguiente: *“Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial”*. La presente solicitud carece de aplicación Industrial ya que como se demostró anteriormente, en el capítulo reivindicatorio se reclama un métodos terapéutico y estos no son aplicables industrialmente porque primero no se obtiene un producto o proceso; y segundo esto no es reproducible o repetible a escala industrial. Por lo tanto, el objeto de la presente invención referido a un método terapéutico no cumple con el requisito de la aplicación industrial.

2.9. Dado lo anterior, es posible deducir que la solicitud y lo reivindicado no es objeto de patentabilidad pues es contrario a lo que dicta la Decisión 486 de la Comunidad Andina de Naciones, Artículo 14.- *“Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial.”*. La solicitud pretendida no cumple con dos de los tres requisitos de patentabilidad.

2.10. Por todo lo anterior, atentamente solicito a usted que se sirva declarar fundada la presente oposición y en consecuencia negar el registro a la solicitud de patente titulada **“USO DE FLIBANSERINA PARA EL TRATAMIENTO DE TRASTORNOS POSMENOPAUSICOS DEL DESEO SEXUAL”**, cuyo titular

es ELAN PHARMACEUTICALS, INC., solicitud Colombiana que apareció publicada en la Gaceta de la Propiedad Industrial N° 613 del 19 de febrero de 2010.

3. PRUEBAS

De conformidad con lo dispuesto por las normas vigentes, solicito a usted tener como prueba en este asunto los siguientes:

- D1: US2005065158 publicada el 24 de marzo de 2005.
- D2: "Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites. Inmunología, volumen 97 páginas 693 a 698 publicado en 1999.

4. FUNDAMENTO DE DERECHO

Fundamento esta observación en las normas legales siguientes:

- 4.1. Numeral 1 del artículo 586 del Código de Comercio.
- 4.2. Artículo 42 de la Decisión 486 de la Comisión del Acuerdo de Cartagena.
- 4.3. En las demás normas concordantes.



5. ANEXOS

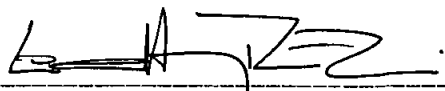
Para los efectos correspondientes anexo al presente escrito los siguientes documentos:

1. Poder para actuar en el presente.
2. Documento que acredita al representante legal de **PROCAPS S.A.**
3. Recibo de caja del respectivo pago de tasa.
4. Copias de los documentos:
 - D1: US2005065158 publicada el 24 de marzo de 2005. D1: US2005065158 publicada el 24 de marzo de 2005.
 - D2: "Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites. Inmunología, volumen 97 páginas 693 a 698 publicado en 1999.

6. NOTIFICACIONES

Para efectos de notificaciones que por disposición del artículo 50 del Decreto 01 de 1984 debe hacer su Despacho, informo que mi representada y la suscrita recibiremos notificaciones en la Secretaría de su Despacho o en mis oficinas situadas en la Carrera 13 N° 119-95, Of. 104 de esta ciudad de Bogotá.

Señor Superintendente,



CIELO ANGELA PEÑA RODRIGUEZ
C.C. 51'940.252 de Bogotá
T.P.A. 82701 del C.S. de la J.



PODER ESPECIAL

Yo, **WALTER TANAKA TATEKAWA**, mayor de edad y vecino de la ciudad de Barranquilla (Colombia), identificado como aparece al pie de mi firma, quien en el presente documento actúa en calidad de Representante Legal de la sociedad **PROCAPS S.A.** sociedad legalmente constituida y domiciliada en la ciudad de Barranquilla (Colombia), por el presente documento otorgo a la doctora **CIELO ANGELA PEÑA RODRIGUEZ**, abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. 82701 del C.S. de la J., poder especial, amplio y suficiente, para formular oposiciones u observaciones a la solicitud de patente No. 08.105.676, titulada **"MÉTODO PARA TRATAR ENFERMEDADES INFLAMATORIAS Y AUTOINMUNES CON NATALIZUMAB"**, cuyo titular es **ELAN PHARMACEUTICALS, INC.**, publicada en la gaceta No. 613 del 19 de febrero de 2010.

Nuestro apoderado queda facultado, de manera expresa, para realizar todo tipo de actos necesarios para llevar a cabo el trámite de la oposición, incluyendo, sin limitación, preparar y presentar solicitudes, hacer declaraciones, pagar tasas, contribuciones e impuestos, recabar los títulos o certificados. Asimismo, quedan autorizados para impugnar administrativa todo lo que fuere resuelto en el expediente a que se hace expresa referencia en el presente poder, con todas las facultades generales y específicas que fuera requerido para ella.

De la misma manera, además de las facultades que le confiere la naturaleza del presente mandato, se encuentran facultados para recibir, desistir, transigir, sustituir, y reasumir el presente poder, incoar acciones, interponer recursos y demás facultades conferidas por la ley.

Dado y Firmado en Barranquilla (Colombia) a los 11 días del mes de Mayo de 2010.

PROCAPS S.A.

C.C. N° 16'251.923
NIT: 890.106.527-5
Representante Legal

Acepto:

CIELO ANGELA PEÑA RODRIGUEZ
C.C. 51'940.252 de Bogotá
T.P.A. 82701 del C.S. de la J.

NOTARIA
NOTARIA TERCERA DEL CIRCULO DE BARRANQUILLA
DILIGENCIA DE RECONOCIMIENTO
Ante el Notario Tercero de Barranquilla se presento:
Walter Turaka Takara
Identificado con: C.C. 16 251 922 Palmira
Y declaro que el contenido del anterior Documento es cierto
y suya la firma que lo ratifica.
Barranquilla: 14 MAYO 2010

[Handwritten signature]

[Handwritten signature]





***** Pag. 1
C A M A R A D E C O M E R C I O
D E B A R R A N Q U I L L A
06*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

EN JUNIO DE ESTE AÑO SE ELEGIRÁ JUNTA DIRECTIVA DE LA CÁMARA DE COMERCIO. LAS INSCRIPCIONES DE CANDIDATOS DEBEN HACERSE EN LA PRIMERA QUINCENA DE MAYO. PARA INFORMACIÓN DETALLADA DIRIGIRSE A LA SEDE PRINCIPAL O COMUNICARSE AL SIGUIENTE TELEFONO: 3303718-3303716.

EL SUSCRITO SECRETARIO DE LA CAMARA DE COMERCIO DE BARRANQUILLA, CON FUNDAMENTO EN LAS INSCRIPCIONES DEL REGISTRO MERCANTIL:

C E R T I F I C A

Que por Escritura Pública No. 1,190 del 22 de Julio de 1976, otorgada en la Notaria Tercera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 28 de Julio de 1976 bajo el No. 6,070 del libro respectivo, fue constituida la sociedad-----
limitada denominada "PRODUCTORA DE CAPSULAS DE GELATINAS LIMITADA
"PROCAPS LIMITADA".-----

C E R T I F I C A

Que por Escritura Pública No. 1,307 del 28 de Junio de 1979, otorgada en la Notaria Tercera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 23 de Julio de 1979 bajo el No. 10,039 del libro respectivo, la sociedad antes mencionada-----
se transformo en sociedad anonima bajo la denominacion social de
"PRODUCTORA DE CAPSULAS DE GELATINA S.A." PROCAPS S.A."-----

C E R T I F I C A

Que por Escritura Pública No. 3,810 del 31 de Dic/bre de 1980, otorgada en la Notaria Primera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 30 de Enero de 1981 bajo el No. 12,705 del libro respectivo, la sociedad antes mencionada-----
se transformo en sociedad de responsabilidad limitada bajo al razon
social de "PRODUCTORA DE CAPSULAS DE GELATINA LIMITADA "PROCAPS LI -
MITADA".-----

C E R T I F I C A

Que por Escritura Pública No. 3,755 del 10 de Nov/bre de 1983, otorgada en la Notaria Unica de Soledad, inscrito(as) en esta Cámara de Comercio, el 22 de Nov/bre de 1983 bajo el No. 17,922 del libro respectivo, la sociedad antes mencionada-----
se transformo en sociedad anonima bajo la denominacion social de
"PRODUCTORA DE CAPSULAS DE GELATINA S.A." PROCAPS S.A."-----

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----
NIT: 890.106.527-5.

C E R T I F I C A

Que por Escritura Pública No. 3,615 del 06 de Dic/bre de 1996, otorgada en la Notaria 2a. de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 20 de Dic/bre de 1996 bajo el No. 67,218 del libro respectivo, la sociedad antes mencionada-----
cambio de razon social, por la denominacion LABORATORIOS PROCAPS S.A

C E R T I F I C A

Que por Escritura Pública No. 3,775 del 18 de Dic/bre de 1996, otorgada en la Notaria 2a. de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 23 de Enero de 1997 bajo el No. 67,704 del libro respectivo, la sociedad antes mencionada-----
cambio de razon social, por la denominacion PROCAPS S.A. -----

C E R T I F I C A

Que dicha sociedad ha sido reformada por las siguientes escrituras y/o documentos privados:

Numero	aaaa/mm/dd	Notaria	No. Insc o Reg	aaaa/mm/dd
39	1979/01/18	Notaria 3a. de Barranquilla.	9,452	1979/01/24
992	1981/04/30	Notaria 1a. de Barranquilla.	13,112	1981/05/06
1,464	1982/08/27	Notaria Unica de Soledad	15,533	1982/09/08
2,744	1983/08/18	Notaria Unica de Soledad	17,416	1983/08/26
3,826	1984/07/16	Notaria Unica de Soledad	19,554	1984/07/27
3,835	1985/08/26	Notaria Unica de Soledad	22,230	1985/09/04
2,260	1986/09/10	Notaria Unica de Soledad	24,995	1986/09/15
1,849	1989/07/11	Notaria 2a. de Barranquilla.	33,909	1989/07/13
444	1990/02/21	Notaria 2a. de Barranquilla.	36,101	1990/03/16
3,090	1991/12/17	Notaria 2a. de Barranquilla	43,993	1992/01/28
269	1993/02/01	Notaria 2a. de Barranquilla	48,428	1993/02/16
2,342	1993/07/28	Notaria 2a. de Barranquilla	50,582	1993/08/10
3,652	1993/11/09	Notaria 2a. de Barranquilla	51,798	1993/11/22
3,615	1996/12/06	Notaria 2a. de Barranquilla	67,218	1996/12/20
89	1997/01/16	Notaria 2a. de Barranquilla	67,704	1997/01/23
2,070	1997/07/17	Notaria 2a. de Barranquilla	70,627	1997/07/31
2,281	1997/08/04	Notaria 2a. de Barranquilla	70,813	1997/08/14
765	1999/04/22	Notaria 3a. de Barranquilla	80,628	1999/04/23
459	2001/02/21	Notaria 2a. de Barranquilla	91,502	2001/02/23
2,608	2001/10/05	Notaria 2a. de Barranquilla	95,771	2001/11/08
1,730	2004/07/29	Notaria 2. de Barranquilla	112,999	2004/08/26
416	2005/03/03	Notaria 2. de Barranquilla	116,364	2005/03/10
1,613	2005/07/28	Notaria 2. de Barranquilla	119,322	2005/08/18
710	2009/05/05	Notaria 3 a. de Barranquilla	150,130	2009/06/30
1,054	2009/06/25	Notaria 3 a. de Barranquilla	150,130	2009/06/30

C E R T I F I C A

Que de acuerdo con la(s) escritura(s) arriba citada(s), la sociedad se rige por las siguientes disposiciones:
DENOMINACION O RAZON SOCIAL:



***** Pag. 2

CAMARA DE COMERCIO
DE BARRANQUILLA

06*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

PROCAPS S.A.-----

SIGLA: PROCAPS.

DOMICILIO PRINCIPAL: Barranquilla.

NIT No: 890.106.527-5.

MATRICULA MERCANTIL: 24,802.

C E R T I F I C A

Direccion para notificaciones judiciales:

CL 80 No 78 B - 201.

De la ciudad de Barranquilla.

C E R T I F I C A

DURACION: El término de duración de la sociedad se fijó hasta el 18 de Agosto de 2073.

C E R T I F I C A

OBJETO SOCIAL: El objeto principal de la sociedad sera: a) La fabricacion, importacion y comercializacion de alimentos procesados y suplementos dietarios. b) La fabricacion de capsulas y otros productos similares a partir de materiales tales como gelatinas u otros de distinta naturaleza, que puedan ser utilizados como envases o recipientes de productos y sustancias quimicas, farmaceuticas, alimenticias y cosmeticas, para uso y consumo humano y veterinario. c) La investigacion, desarrollo y fabricacion de toda clase de productos y sustancias quimicas, farmaceuticas, alimenticias y cosmeticas para uso y consumo humano, animal y vegetal. d) La compra y venta de materias primas, insumos y productos intermedios necesarios para los procesos de fabricacion de sustancias quimicas, farmaceuticas, alimenticias y cosmeticas, asi como la comercializacion interna y externa de los productos terminados. e) La compra, venta, importacion, exportacion y distribucion de toda clase de elementos instrumentales y equipos medicos, quirurgicos, odontologicos y hospitalarios para la salud humana y animal. f) La compra, venta, exportacion, fabricacion, importacion, distribucion de dulces, confites, galletas y todo tipo de golosinas o alimentos para el consumo humano. g) Actuar como representante o agente de personas naturales y juridicas, nacionales y extranjeras en la realizacion de operaciones o transacciones relacionadas con la industria farmaceutica y en especial con cualquiera de las actividades descritas en los literales a), b), c), d) y e) antes descritos. h) Prestar los servicios de asesorias y consultoria en las areas administrativas,

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de mercadeo, analisis clinico, asistencia tecnica, investigacion, costos y produccion, exportaciones, importaciones, ventas, relacionadas con iguales o similares actividades a las que constituyen el objeto de PROCAPS S.A., asi como los de publicidad y promocion de productos quimicos, alimenticios, cosmeticos y farmaceuticos para uso humano, animal o industrial. i) Adquirir, operar, administrar y explotar, a cualquier titulo, empresa o establecimientos dedicados a la investigacion, desarrollo, fabricacion o comercializacion de productos quimicos, farmaceuticos, alimenticios, hospitalarios o cosmeticos. En desarrollo de su objeto principal, la sociedad podra tomar interes social como accionista, socio o fundador de empresas que tengan igual o similar objeto, comprar, vender, importar y exportar maquinarias, insumos y repuestos requeridos para el desarrollo de sus actividades; tomar dinero en prestamo, financiar y garantizar las operaciones comerciales que celebre con terceros relativas a su objeto; celebrar y ejecutar todo tipo de actos y contratos necesarios para desarrollar sus operaciones; girar, aceptar, otorgar y protestar todo tipo de titulos valores e instrumentos negociables, comprar, vender y constituir gravámenes sobre bienes muebles e inmuebles, designar representantes apoderados en el pais y en el exterior y, en general, realizar cualquier acto o negocio que sea necesario o conveniente para el desarrollo de su objeto social y para la mejor proteccion de sus intereses corporativos o la de sus socios. La sociedad podra garantizar el cumplimiento de sus obligaciones adquiridas por terceros o por sus socios, para lo cual podra inclusive constituir gravámenes reales sobre sus bienes de cualquier naturaleza.

DE BARRANQUILLA

C E R T I F I C A

CAPITAL	Nro Acciones	Valor Acción
Autorizado		
\$*****2,000,000,000	*****2,000,000	*****1,000
Suscrito		
\$*****1,229,071,000	*****1,229,071	*****1,000
Pagado		
\$*****1,229,071,000	*****1,229,071	*****1,000

C E R T I F I C A

ADMINISTRACION: La direccion y administracion de la sociedad corresponde a la Asamblea General de Accionistas, a la Junta Directiva, al Presidente, al Vicepresidente y a un Gerente General. Corresponde a la Junta Directiva las siguientes funciones y atribuciones entre otras: Autorizar cualquier acto o contrato cuya cuantia sea superior al equivalente en pesos Colombianos a doscientos cincuenta mil dolares de los Estados Unidos de America (US\$250.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la transacion. Cuando se trate de operaciones en virtud de las cuales la sociedad garantice obligaciones de terceros o de los socios, se requerira que la operacion sea aprobada con el voto de por lo menos 2/3 partes de los integrantes de la Junta Directiva.

***** C O N T I N U A *****



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06*****

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La sociedad tendra un Presidente , quien ejercera la representacion legal de la sociedad y tendra las siguientes atribuciones entre otras: Ejecutar las decisiones de la Asamblea General de Accionistas y de la Junta Directiva. Ejercer la Representacion Legal de la sociedad en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Constituir apoderados judiciales y extrajudiciales, recibir notificaciones, absolver interrogatorios de parte, confesar obligaciones de la sociedad, transigir, disistir y allanar a la sociedad en cualquier asunto judicial. Aprobar y celebrar en nombre de la socieda todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos colombianos a doscientos cincuenta mil dolares de los Estados Unidos de America (US\$250.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la transacion o celebracion del acto. Las demas funciones que le correspondan como Representante Legal por disposicion de la ley, de estos estatutos o de la Junta Directiva. El Presidente tendra un suplente, designado por la Junta Directiva, quien reemplazara al Presidente en sus faltas temporales, absolutas o meramente accidentales, con entidades facultades y sin que sea necesario para ello acreditar la ausencia del principal. La sociedad tendra un Vicepresidente designado por la Junta Directiva, quien tambien ejercera la representacion legal de la sociedad, pudiendo ejercerla conjunta o separadamente con el Presidente. El vicepresidente tendra las siguientes facultades entre otras. Ejecutar dentro de su competencia las decisiones de la Asamblea General de Accionistas, de la Junta Directiva y del Presidente. Ejercer la representacion legal de la sociedad en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Constituir apoderados judiciales y extrajudiciales, recibir notificaciones, absolver interrogatorios de parte, confesar obligaciones de la sociedad, transigir, desistir y allanar a la sociedad en cualquier asunto judicial. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos colombianos a ciento cincuenta mil dolares de los Estados Unidos de America (US\$150.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la aprobacion o celebracion del acto, siempre que no se trate de enajenar bienes inmuebles o cualquier otro activo fijo de la sociedad, inclusive de naturaleza muebles, acciones, cuotas o partes de interes inversiones y todo tipo de derechos de la sociedad, o de constituir gravámenes sobre cualquiera de los anteriores, facultades que radican exclusiva y privativamente en cabeza del Presidente. Las demas funciones que le corresponda como

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Representante legal por disposicion de la ley, de estos estatutos o de la Junta Directiva. El Vicepresidente tendra (1) suplente, designado por la Junta Directiva, quien lo reemplazara conjunta o separadamente en sus faltas temporales, absolutas o meramente accidentales, con identicas facultades y sin que sea necesario para ello acreditar la ausencia del principal. La sociedad tendra un (1) Gerente General, con su respectivo Suplente, designados por la Junta Directiva. El Suplente reemplazara al Gerente General, en sus faltas temporales, absolutas o meramente accidentales, quien junto con el gerente general, llevaran la representacion legal de la sociedad, con identicas facultades, pudiendolas ejercer conjunta o separadamente con el Presidente y Vicepresidente, con identicas facultades que el Gerente General y sin que sea necesario para ello acreditar la ausencia del titular. El Gerente General tendra las siguientes facultades entre otras: Ejercer la representacion legal de la sociedad, en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos Colombianos a Cincuenta Mil dolares de los Estados Unidos de America (US\$50.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la aprobacion o celebracion del acto, siempre que no se trate de enajenar a cualquier titulo bienes inmuebles o cualquier otro activo fijo de la sociedad, inclusive de naturaleza mueble, acciones, cuotas o partes de interes, inversiones y todo tipo de derechos de la sociedad o de constituir gravámenes sobre cualquiera de las anteriores facultades que radican exclusiva y privativamente en cabeza del Presidente.

C E R T I F I C A

Que según Acta No. 116 del 27 de Junio de 1997 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad:

PROCAPS S.A.-----

cuya parte pertinente se inscribió en esta Cámara de Comercio, el 15 de Agosto de 1997 bajo el No. 70,838 del libro respectivo, y según Acta No. 127 del 22 de Marzo de 2000 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad:

PROCAPS S.A.-----

cuya parte pertinente se inscribió en esta Cámara de Comercio, el 29 de Mayo de 2000 bajo el No. 87,250 del libro respectivo, fueron hechos los siguientes nombramientos:

CLASE:

JUNTA DIRECTIVA

Principales

1. Minski Gontovnik Ruben
2. Minski Gontovnik Meyer
3. Minski Gontovnik Jose

CC.*****7,463,982

CC.*****7,461,324

CC.*****8,671,834

Suplentes

1. Minski Zirberblum Elliot

CC.*****72,219,541

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2. Minski Deitel Daniel

3. Minski Sharon

PA.*990,212,082,805

C E R T I F I C A

Que según Acta No. 116 del 27 de Junio de 1997 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 15 de Agosto de 1997 bajo el No. 70,838 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre

Identificación

Presidente.

Minski Gontovnik Ruben

CC. *****7,463,982

Suplente del Presidente

Minski Minski Abraham

CC.*****802,575

Vicepresidente

Minski Gontovnik Meyer

CC.*****7,461,324

Suplente del vicepresidente

Minski Gontovnik Jose

CC. *****8,671,834

C E R T I F I C A T E

Que según Acta No. 11 del 01 de Febrero de 2001 correspondiente a la Junta Directiva en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 12 de Febrero de 2001 bajo el No. 91,273 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre

Identificación

Gerente General.

Uribe Ochoa Guillermo Luis

CC.*****70,073,754

C E R T I F I C A

Que según Acta No. 406 del 02 de Sep/bre de 2004 correspondiente a la Junta Directiva en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 09 de Sep/bre de 2004 bajo el No. 113,263 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre

Identificación

Suplente del Gerente.

Tanaka Tatekawa Walter

CC.*****16,251,923

C E R T I F I C A

Que según Acta No. 124 del 29 de Marzo de 1999 correspondiente a

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PROCAPS S.A.-----

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la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 04 de Agosto de 1999 bajo el No. 82,226 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Revisor Fiscal Ppal.	
Vargas Pertuz Ana Isabel	CC.*****32,696,645

C E R T I F I C A

Que según Acta No. 151 del 31 de Marzo de 2009 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 30 de Julio de 2009 bajo el No. 151,129 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Suplente del Revisor Fiscal.	
Duarte Diaz David	CC.*****73,115,965

C E R T I F I C A

Que por Escritura Pública No. 2,784 del 07 de Dic/bre de 2007, otorgada en la Notaria 2 a. de Barranquilla cuya parte pertinente se inscribió en esta Cámara de Comercio, el 20 de Dic/bre de 2007 bajo el No. 3,492 del libro respectivo y las inscripciones 3.492-1, 3.493, 3.494, 3.495, 3.496, 3.497, 3.498, 3.499, 3.500, 3.501, 3.502, 3.503, 3.504, 3.505, 3.506, 3.507, Consta que RUBEN MINSKI GONTOVNIK, C.C. No. 7.463.982, obrando como Presidente y Representante Legal de la sociedad C.I. NATURMEGA S.A., que obrando en su condicion de Presidente y Representante Legagl de la sociedad PROCAPS S.A., el presidente debidamente facultado por los Estatutos Sociales y por el Maximo Organó Social, otorga los siguientes poderes: Otorgar poder general, amplio y suficiente a los doctores JOSEFINA DEL CARMEN MIELES BUELVAS CC.No.33.138.497; a MARCELA CARVAJALINO PAGANO CC.No.22.579.748; CRISTINA VIOLI FRANCESCHINI CC.No.22.463.716, con las facultades que se transcriben a continuacion: a.- Para que representen a la sociedad PROCAPS S.A, ante todo tipo de autoridades administrativas y jurisdiccionales, en asuntos de naturaleza civil, laboral, comercial, penal, aduanera, cambiaria, tributaria, policiva, administrativa y fiscal. b.- Contestar y presentar demandas, requerimientos, recursos, pedir y aportar pruebas, recibir, notificarse, ejercer la accion de tutela, tramitar incidente de desacato, comparecer en los procesos que cursen contra la sociedad, allanarse en nombre de la sociedad en los asuntos que sean necesarios, transigir, desistir, sustituir, renunciar y reasumir el poder. c.- La facultad expresa de conciliar, por tanto pueden llegar a acuerdos conciliatorios, transaccionales, judiciales o extrajudiciales o extrajudiciales, cualquiera que sea su cuantia, para lo cual las apoderadas deben aportar la autorizacion simple y escrita del Representante legal de la sociedad. d.- Podran presentar solicitudes de devolucion y/o compensacion de impuestos, ventas y rentas, recibir los pagos por

***** C O N T I N U A *****



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las devoluciones y adelantar todo tramite o proceso administrativo ante la DIAN, de tal forma que en ningun momento quede sin ----- representacion legal de la sociedad. PODERES ESPECIALES: a.- Se ---- confiere poder especial amplio y suficiente a las personas que se determinan seguidamente, que conforman el grupo A y B, para, para que conjuntamente suscriban contratos de suministros de mercancías y servicios con entidades oficiales, hasta la suma de OCHOCIENTOS MIL DOLARES DE LOS ESTADOS UNIDOS DE AMERICA (US800.000.00), ----- liquidados a la tasa representativa del mercado vigente en el día que se realice la respectiva transaccion. Grupo A. WALTER NANAKA TATEKAWA CC.No.16.251.923, CLAUDIA TANGARIFE CASTILLO ----- CC.No.41.660.293, CARMEN ALICIA FLOREZ CC.No.22.434.044, IVAN ----- VILLAREAL CAMACHO CC.No.91.201.749, CARMEN ALICIA HERRERA ----- DIAZGRANADOS CC.No.22.377.540, DAVID ANTONIO CHARRIS GIRALDO ----- CC.No.19.341.274, RICARDO DORADO HURTADO CC.No.79.715.772, JAIME FORERO LLINAS CC.No.19.300.593. b.- Se confiere poder especial ---- amplio y suficiente al señor WILLIAM ALBERTO BARROS CERRA ----- CC.No.8.715.941, para que en nombre y representación de PROCAPS ---- S.A., suscriban declaraciones cambiarias, aduaneras y tributarias a que este obligada la sociedad, en el giro normal de sus negocios y operaciones sociales, por tanto quedan facultados para firmarlas, como tambien los demás documentos relacionados con ellas, ---- notificarse, aportar y solicitar las pruebas que sean necesarias. c.- Otorgar poder especial amplio y suficiente al señor MARLON ----- TATIS PAREDES CC.No.72.176.874 y al señor WILLIAM ALBERTO BARROS CERRA CC.No.8.715.941, para que en nombre y representación de ----- PROCANPS S.A., suscriban las declaraciones, reportes y cualquier documento relacionado con los tramites o registros de Comercio ----- Exterior y o cualquier entidad del Estado que haga sus veces: d) Otorgar poder especial amplio y suficiente a la doctora CARMEN ----- ALICIA HERRERA DIAZGRANADOS, CC.No.22.377.540 y al doctor WALTER TANAKA TATEKAWA, CC.No.16.251.923, para que en nombre y ----- representación de PROCAPS S.A.; endosen los titulos valores de la sociedad hasta por la suma de UN MIL MILLONES DE PESOS ----- (1.000.000.000.00) M.L. CUARTO: EI representante legal de la ----- sociedad PROCAPS S.A.; doctor RUBEN MINSKI GONTOVNIK, manifiesta que la presente reforma fue aprobada por la Asamblea General ----- Extraordinaria de Accionista de PROCAPS S.A., con observancia de las disposiciones legales y estatutarias.-----

C E R T I F I C A

Que por Escritura Pública No. 1,933 del 16 de Sep/bre de 2008, otorgada en la Notaria 2 a. de Barranquilla cuya parte pertinente se inscribió en esta Cámara de Comercio, el 06 de Octubre de 2008

***** C O N T I N U A *****

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bajo el No. 3,748 del libro respectivo,-----
Consta que el señor RUBEN MINSKI GONTOVNIK, C.C. No. 7.463.982, que
comparece en su condicion de Presidente y Representante Legal de la
Sociedad PROCAPS S.A., confiere por medio de este instrumento -----
publico Poder especial Amplio y Suficiente a la doctora MILENA -----
ESCAFFI PARDO, C.C. No. 32.758.420, para que en nombre y -----
representacion de PROCAPS S.A., suscriba declaraciones cambiarias,
aduaneras y tributarias a que este obligada la sociedad en el giro
normal de sus negocios y operaciones sociales, por tanto queda -----
facultada para firmar, como tambien los demas documentos -----
relacionados con ellas, notificarse, aportar y solicitar las -----
pruebas que sean necesarias.-----

C E R T I F I C A

Que por Documento Privado del 09 de Abril de 2010, otorgado en
Barranquilla inscrito en esta Cámara de Comercio, el 20 de Abril de
2010 bajo el Nro 4,201 del libro respectivo,-----
Consta que el señor RUBEN MINSKI GONTOVNIK, C.C. No. 7.463.982, ----
quien obra en el caracter de Presidente y/o Representante legal de
la sociedad PROCAPS S.A., que por medio de este instrumento -----
publico, otorga Poder Especial Amplio y Suficiente a la Doctora ----
MONICA DE HART DE OCHOA, identificada con C.C. No. 32.676.025, para
que en nombre y representacion de la sociedad, Endose las Facturas
Nacionales e Internacionales hasta por la suma de UN MIL MILLONES DE
PESOS (\$1.000.000.000.00)M.L. La doctora Monica de Hart de Ochoa,
queda facultada para realizar todo acto o tramite necesario para el
cabal cumplimiento del presente mandato.-----

C E R T I F I C A

Que en esta Cámara de Comercio no aparecen inscripciones posteriores
de documentos referentes a reforma, disolución, liquidación o
nombramientos de representantes legales de la expresada sociedad.

C E R T I F I C A

Que su última Renovación fue el: 30 de Marzo de 2010.

La información sobre embargos de establecimiento se suministra en
Certificados de Matrícula, la de contratos sujetos a registro, en
Certificados Especiales.

C E R T I F I C A

Que los actos de registro aquí certificados quedan en firme cinco
días hábiles despues de la correspondiente inscripción, siempre
que, dentro de dicho término, no sean objeto de los recursos de
reposición ante esta entidad, y/o de apelación para ante la
Superintendencia de Industria y Comercio.

03-12010500 024802 PROCAPS S.A.
Barranquilla, 04 de Mayo de 2010

Hr:09:24:52

No. 14610510



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mpayson



SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 08-105676- -00003-0000

Fecha: 2010-05-25 14:34:07 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 400 OPOSIOBSERVTER Folios: 66

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 10 - 42,372
FECHA : MAYO 25 DE 2010

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
LAB. PROCAPS	CONSIGNACION	BANCO DE BOGOTA	062754387	353106810	25/05/2010	885,000.00
LAB. PROCAPS	CONSIGNACION	BANCO DE BOGOTA	062754387	353106821	25/05/2010	2,065,000.00

***** CONCEPTO *****

CANT. RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1 50005-01-04 PRESENTACION DE OBSERVACIONES	12 MARCAS Y LEMAS COMERCIALES	295,000.00
	TOTAL :	\$ 295,000.00

SON: DOSCIENTOS NOVENTA Y CINCO MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____



US 20040009169A1

(19) **United States**

(12) **Patent Application Publication**
Taylor et al.

(10) Pub. No.: **US 2004/0009169 A1**
(43) Pub. Date: **Jan. 15, 2004**

(54) **ADMINISTRATION OF AGENTS FOR THE TREATMENT OF INFLAMMATION**

Publication Classification

(76) Inventors: **Julie Taylor**, San Francisco, CA (US);
Theodore A. Yednock, Forest Knolls, CA (US)

(51) Int. Cl.⁷ **A61K 39/395**
(52) U.S. Cl. **424/144.1**

Correspondence Address:
BURNS DOANE SWECKER & MATHIS L L P
POST OFFICE BOX 1404
ALEXANDRIA, VA 22313-1404 (US)

(57) **ABSTRACT**

(21) Appl. No.: **10/372,111**

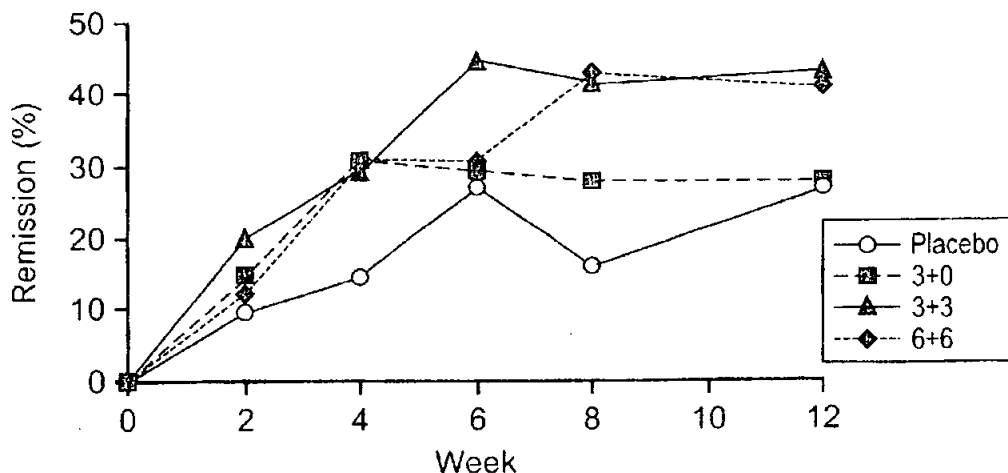
(22) Filed: **Feb. 25, 2003**

Related U.S. Application Data

(60) Provisional application No. 60/374,501, filed on Apr. 23, 2002. Provisional application No. 60/360,134, filed on Feb. 25, 2002.

A method of chronically reducing a patient's pathological inflammation via the administration of an agent that specifically binds to an alpha-4 integrin or a dimer comprising an alpha-4 integrin is disclosed. The agent provided must have a binding affinity such that administration is sufficient to suppress pathological inflammation, and the agent is administered chronically to provide long-term suppression of pathological inflammation.

Remission in the CD Study



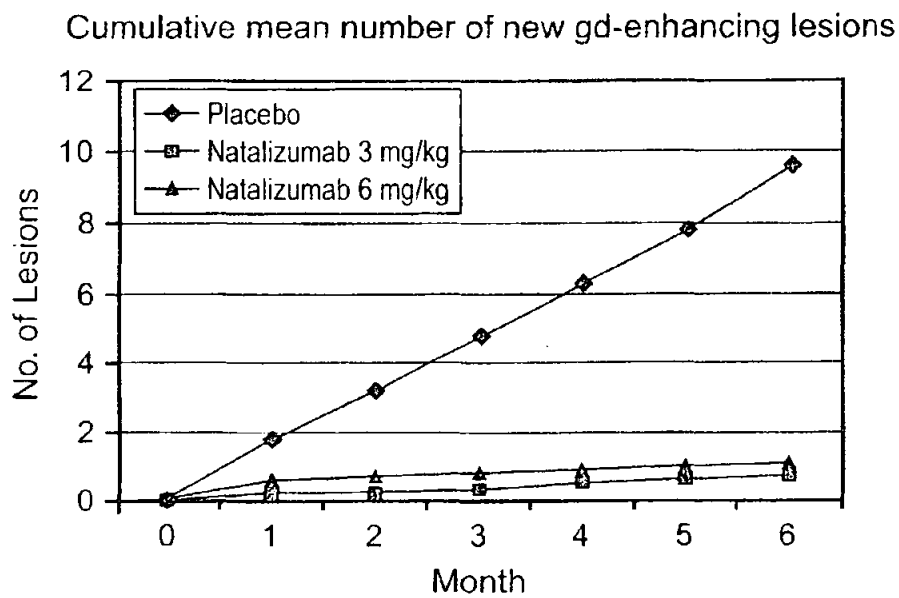


FIG. 1

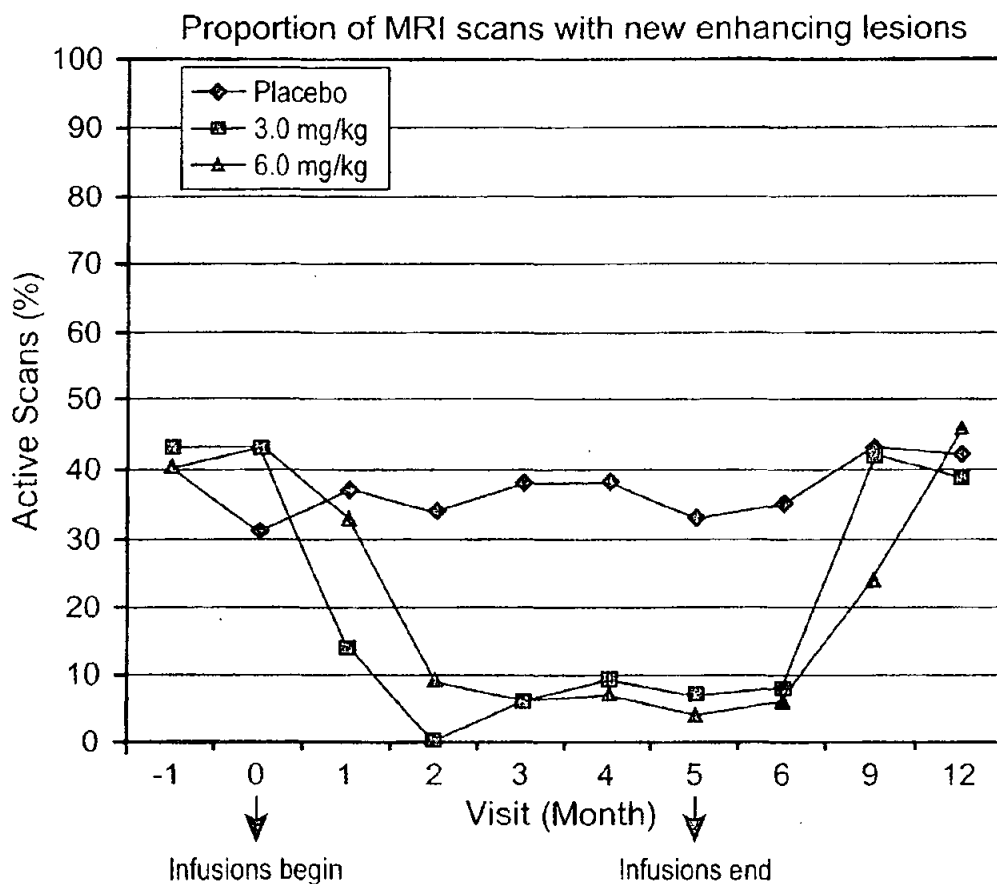


FIG. 2

Summary of Serum Natulizimab Concentrations for 3.0 mg/kg Natulizimab

Patient	Month 0					Month 1					Month 2					Month 3										
	Pre-infusion	60 min.	2 hrs	24 hrs		Pre-infusion	60 min.	2 hrs	24 hrs		Pre-infusion	60 min.	2 hrs	24 hrs		Pre-infusion	60 min.	2 hrs	24 hrs		Pre-infusion	60 min.	2 hrs	24 hrs		
01	<0.13	65.0	74.7	43.4	μg/ml	11.6	μg/ml	17.7	11.2	μg/ml	4.9	μg/ml	11.2	4.6	μg/ml	ND	4.9	μg/ml	11.2	4.6	μg/ml	2.3	μg/ml	11.2	4.6	μg/ml
02	<0.13	56.1	72.2	39.2		17.7		10.6	5.8		ND		5.8	7.2		3.6		7.2	10.7		3.6		1.3		1.3	
03	<0.13	58.5	60.0	34.4		10.6		16.2	54.1		3.6		7.2	10.7		2.3		8.0	4.0		2.3		0.2		0.2	
04	<0.13	77.9	97.3	54.1		16.2		17.3	42.0		8.0		10.7	4.0		1.5		1.5	ND		1.5		5.4		5.4	
05	<0.13	75.6	67.5	42.0		17.3		10.7	ND		1.5		4.0	ND		ND		ND	ND		ND		1.6		1.6	
06	<0.13	72.0	73.7	23.7		13.0		15.7	23.7		6.7		9.9	9.9		3.5		6.7	9.9		3.5		4.0		4.0	
07	<0.13	16.9	30.7	49.0		15.7		18.6	49.0		3.5		ND	ND		2.2		3.5	ND		2.2		2.2		2.2	
08	<0.13	54.3	58.4	62.6		18.6		22.7	62.6		7.3		10.7	10.7		3.9		7.3	10.7		3.9		3.9		3.9	
09	<0.13	82.7	74.2	42.0		14.3		25.5	42.0		4.1		6.8	6.8		2.2		4.1	6.8		2.2		2.2		2.2	
10	<0.13	84.3	84.1	82.1		25.5		13.7	82.1		5.8		13.7	13.7		3.5		5.8	13.7		3.5		3.5		3.5	
11	<0.25	62.5	72.6	44.3		13.7		11.0	44.3		5.3		8.6	8.6		2.7		5.3	8.6		2.7		2.7		2.7	
12	<0.25	102.7	80.6	36.5		11.0			36.5		3.9		6.3	6.3		2.0		3.9	6.3		2.0		2.0		2.0	
13	<0.13	76.0	67.8																							
14	<0.13	66.4	70.2																							
Month 0																										
Pre-infusion 60 min. 2 hrs 24 hrs Week 1 Week 2 Week 3 Month 1																										
N	14	14	14	13		14		14	12		12		12	12		14		12	12		14		14		14	
Mean	0.0	67.9	70.3	47.5		15.6		15.6	8.3		8.3		8.3	8.3		2.7		4.7	4.7		2.7		2.7		2.7	
SD	0.00	19.60	14.96	15.18		4.50		4.50	2.98		2.98		2.98	2.98		1.33		1.97	1.97		1.33		1.33		1.33	

FIG. 3A

Summary of Serum Natulizimab Concentrations for 3.0 mg/kg Natulizimab

Month 5

Patient	Month 2	Month 3	Month 4	Pre-infusion	60 min.	2 hrs	24 hrs
01	2.4 µg/ml	1.4 µg/ml	1.9 µg/ml	ND	ND	ND	ND
02	7.1	8.0	11.1	7.8	65.1	82.3	50.5
03	2.3	2.9	2.7	1.2	53.9	54.7	40.6
04	<0.13	<0.13	<0.13	<0.13	24.6	21.4	2.5
05	8.1	11.4	1.8	0.2	ND	ND	ND
06	1.4	1.8	1.9	2.0	81.9	90.3	51.9
07	1.7	2.5	2.2	2.6	36.0	62.7	22.2
08	4.5	5.7	2.0	4.7	76.0	65.0	ND
09	3.4	3.9	3.3	3.3	59.7	64.8	48.5
10	2.9	3.3	6.1	4.5	82.0	79.6	59.9
11	2.7	4.6	5.5	3.0	82.6	79.3	55.6
12	2.0	1.9	1.9	3.6	68.8	ND	65.2
13	4.3	4.3	3.6	5.0	74.9	55.4	47.0
14	2.1	1.6	1.9	3.0	70.3	63.0	35.5

Month 5

SRN

	Month 2	Month 3	Month 4	Pre-infusion	60 min.	2 hrs	24 hrs
N	14	14	14	13	12	11	11
Mean	3.2	3.8	3.3	3.1	64.7	65.3	43.6
SD	2.19	2.98	2.74	2.11	18.51	18.61	18.03

FIG. 3B

Summary of Serum Natulizimab Concentrations for 3.0 mg/kg Natulizimab

Patient	Week 21	Week 22	Week 23	Month 6	Month 7	Month 8	Month 9	Month 10	Month 12
01	ND	ND	ND	ND	ND	ND	ND	ND	ND
02	33.8	21.4	10.3	8.1	0.4	<0.13	<0.13	ND	ND
03	21.3	9.4	4.7	5.1	<0.25	<0.13	<0.13	ND	ND
04	<0.13	<0.13	<0.13	<0.13	<0.25	<0.13	<0.13	ND	ND
05	ND	ND	ND	0.2	<0.25	<0.13	<0.13	ND	ND
06	21.5	7.6	3.9	2.4	<0.13	<0.13	<0.13	ND	ND
07	ND	11.0	6.2	3.7	0.3	<0.13	ND	ND	ND
08	19.1	13.2	9.6	5.3	0.4	<0.13	<0.13	<0.13	ND
09	33.7	13.1	6.1	3.8	<0.13	ND	ND	ND	ND
10	29.9	16.8	ND	7.4	0.5	ND	ND	ND	ND
11	ND	18.4	10.5	6.6	0.7	<0.13	ND	ND	ND
12	ND	9.9	6.3	3.5	<0.13	<0.13	ND	ND	ND
13	22.1	13.3	9.1	4.6	<0.13	<0.13	ND	ND	ND
14	14.1	8.2	9.4	4.7	<0.13	ND	ND	ND	ND
SRN	Week 21	Week 22	Week 23	Month 6	Month 7	Month 8	Month 9	Month 10	Month 12
N	9	12	11	13	13	10	6	1	0
Mean	21.7	11.9	6.9	4.3	0.2	0.0	0.0	0.0	
SD	10.57	5.61	3.25	2.44	0.25	0.00	0.00		

FIG. 3C

Summary of Serum Natulizimab Concentrations for 6.0 mg/kg Natulizimab

Patient	Month 0										Month 1			
	Pre-infusion	60 min.	2 hrs	24 hrs	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Month 1
01	<0.25	104.8	155.3	110.0	36.3	16.8	11.4	36.3	16.8	11.4	36.3	16.8	11.4	11.0
02	<0.13	102.8	135.0	81.8	37.5	18.7	11.0	37.5	18.7	11.0	37.5	18.7	11.0	3.9
03	<0.13	121.1	96.3	68.4	22.9	19.9	11.4	22.9	19.9	11.4	22.9	19.9	11.4	6.6
04	<0.13	136.0	141.2	86.6	26.0	20.9	13.7	26.0	20.9	13.7	26.0	20.9	13.7	9.8
05	<0.13	163.8	138.6	84.4	29.4	17.5	14.0	29.4	17.5	14.0	29.4	17.5	14.0	9.6
06	<0.13	145.7	173.7	94.8	29.3	16.5	14.0	29.3	16.5	14.0	29.3	16.5	14.0	9.7
07	<0.25	173.5	164.0	110.0	33.9	ND	ND	33.9	ND	ND	33.9	ND	ND	ND
08	<0.13	135.8	143.0	105.8	20.9	17.6	15.0	20.9	17.6	15.0	20.9	17.6	15.0	10.9
09	<0.13	97.3	127.9	66.8	27.0	8.3	7.8	27.0	8.3	7.8	27.0	8.3	7.8	4.8
10	<0.13	149.2	111.2	103.2	37.0	21.4	11.9	37.0	21.4	11.9	37.0	21.4	11.9	4.8
11	<0.25	111.6	142.5	88.9	20.8	20.7	9.7	20.8	20.7	9.7	20.8	20.7	9.7	8.6
12	<0.13	147.6	135.0	95.5	43.9	28.0	19.4	43.9	28.0	19.4	43.9	28.0	19.4	9.9
13	<0.25	183.9	168.6	113.2	52.0	31.7	20.9	52.0	31.7	20.9	52.0	31.7	20.9	12.1
14	<0.13	140.2	129.2	109.9	36.9	21.0	12.2	36.9	21.0	12.2	36.9	21.0	12.2	7.9
Month 0														
N	Month 0										Month 1			
	Pre-infusion	60 min.	2 hrs	24 hrs	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Month 1
Mean	14	14	140.1	94.2	32.4	19.9	13.3	32.4	19.9	13.3	32.4	19.9	13.3	8.4
SD	0.00	26.72	21.18	15.38	8.97	5.61	3.63	8.97	5.61	3.63	8.97	5.61	3.63	2.64

FIG. 4A

Summary of Serum Natulizimab Concentrations for 6.0 mg/kg Natulizimab

Patient	Month 5						
	Month 2	Month 3	Month 4	Pre-infusion	60 min.	2 hrs	24 hrs
01	10.7 µg/ml	ND µg/ml	2.4 µg/ml	ND	ND	ND	ND
02	6.8	8.2	9.6	10.3	ND	ND	ND
03	8.6	9.9	10.5	7.8	92.6	97.5	85.9
04	11.8	16.4	17.1	15.8	200.1	161.1	135.6
05	9.3	11.6	16.9	19.5	190.4	168.3	104.4
06	11.7	14.5	16.8	18.2	171.3	153.0	112.7
07	ND	ND	ND	ND	ND	ND	ND
08	14.6	12.9	14.2	18.4	145.2	172.5	99.2
09	8.5	8.4	12.0	ND	103.0	107.5	44.9
10	9.0	16.3	18.1	10.9	114.3	141.4	103.0
11	11.8	14.2	13.3	14.5	135.7	109.4	93.8
12	11.7	ND	ND	ND	ND	ND	ND
13	1.6	<0.13	1.9	7.5	187.0	141.8	124.6
14	11.2	12.6	12.8	11.8	152.4	124.0	114.6
Month 5							
SRN							
	Month 2	Month 3	Month 4	Pre-infusion	60 min.	2 hrs	24 hrs
N	13	11	12	10	10	10	10
Mean	9.8	11.4	12.1	13.5	149.2	137.7	101.9
SD	3.18	4.71	5.39	4.43	37.96	26.82	24.75

FIG. 4B

Summary of Serum Natulizimab Concentrations for 6.0 mg/kg Natulizimab

Patient	Week 21	Week 22	Week 23	Month 6	Month 7	Month 8	Month 9	Month 10	Month 12
01	ND	ND	ND	ND	ND	ND	ND	ND	ND
02	ND	ND	ND	ND	ND	ND	ND	ND	ND
03	43.2	17.2	15.5	10.1	1.3	<0.13	<0.13	ND	ND
04	59.1	38.6	25.1	19.3	3.4	0.2	<0.13	ND	ND
05	46.0	29.3	21.3	24.8	6.5	0.3	0.2	ND	ND
06	78.9	42.9	25.1	17.4	2.9	<0.13	<0.13	ND	ND
07	ND	ND	ND	ND	ND	ND	ND	ND	ND
08	50.1	26.0	24.7	21.2	ND	0.7	<0.13	<0.13	ND
09	32.2	30.2	16.6	9.3	1.1	<0.13	<0.13	ND	ND
10	50.7	34.3	17.0	13.1	2.1	<0.13	<0.13	<0.13	ND
11	34.0	30.0	23.7	22.5	4.6	ND	ND	ND	ND
12	ND	ND	ND	ND	ND	ND	ND	ND	ND
13	48.1	31.9	19.3	ND	ND	ND	ND	ND	ND
14	44.8	32.3	15.5	13.6	2.6	ND	ND	ND	ND
N	10	10	10	9	8	7	7	2	0
Mean	48.7	31.3	20.4	16.8	3.1	0.2	0.0	0.0	
SD	13.20	6.93	4.08	5.56	1.79	0.26	0.08	0.00	

FIG. 4C

Levels of receptor saturation maintained in the MS study					
			Placebo (n=71)	3.0 mg/kg Antegren (n=68)	6.0 mg/kg Antegren (n=74)
Visit	Sampling Time	Statistic			
Month 0	Pre-infusion	N	14	13	14
		Mean	9.82	8.06	7.56
		SD	5.331	2.192	3.135
		Median	8.3	7.7	6.7
		Minimum	4.7	5.9	3.1
Month 0	2-Hr Post infusion	Maximum	24.9	14.4	13.3
		N	14	14	14
		Mean	8.21	99.11	100.97
		SD	3.567	10.557	4.884
		Median	7.5	102.4	102.1
		Minimum	5.2	74.9	93.2
		Maximum	18.6	110.8	110.7
			Placebo (n=71)	3.0 mg/kg Antegren (n=68)	6.0 mg/kg Antegren (n=74)
Visit	Sampling Time	Statistic			
Month 0	24-Hr Post infusion	N	12	12	13
		Mean	9.30	99.09	98.93
		SD	7.328	9.052	12.079
		Median	6.7	99.9	97.4
		Minimum	5.6	81.5	81.2
Week 1		Maximum	31.9	109.4	117.5
		N	14	14	14
		Mean	7.92	93.41	99.61
		SD	2.579	5.656	12.011
		Median	8.3	94.3	94.9
		Minimum	3.5	82.8	83.5
		Maximum	13.3	100.9	120.8

FIG. 5A

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Levels of receptor saturation maintained in the MS study

Visit	Sampling Time	Statistic	Placebo (n=71)		3.0 mg/kg Antegren (n=68)		6.0 mg/kg Antegren (n=74)	
Week 2		N	12	14	13	13	13	13
		Mean	8.98	88.28	97.37	97.37	97.37	97.37
		SD	2.991	7.494	14.377	14.377	14.377	14.377
		Median	8.4	88.0	97.9	97.9	97.9	97.9
		Minimum	4.1	75.9	78.3	78.3	78.3	78.3
Week 3		Maximum	13.4	101.4	131.7	131.7	131.7	131.7
		N	13	13	12	12	12	12
		Mean	8.17	80.99	92.79	92.79	92.79	92.79
		SD	2.242	19.150	9.767	9.767	9.767	9.767
		Median	8.8	85.5	92.3	92.3	92.3	92.3
		Minimum	4.9	24.5	74.7	74.7	74.7	74.7
		Maximum	12.0	99.3	110.9	110.9	110.9	110.9
Month 1		N	14	13	13	13	13	13
		Mean	8.87	82.48	85.58	85.58	85.58	85.58
		SD	3.808	11.272	8.491	8.491	8.491	8.491
		Median	8.4	84.1	84.6	84.6	84.6	84.6
		Minimum	3.1	56.1	68.6	68.6	68.6	68.6
Month 2		Maximum	17.3	100.2	96.1	96.1	96.1	96.1
		N	12	14	13	13	13	13
		Mean	9.41	79.15	93.21	93.21	93.21	93.21
		SD	3.846	22.779	9.194	9.194	9.194	9.194
		Median	9.4	87.1	94.0	94.0	94.0	94.0
		Minimum	4.7	7.3	75.8	75.8	75.8	75.8
		Maximum	19.5	96.6	104.2	104.2	104.2	104.2

FIG. 5B

Levels of receptor saturation maintained in the MS study

Visit	Sampling Time	Statistic	Placebo (n=71)		3.0 mg/kg Antegren (n=68)		6.0 mg/kg Antegren (n=74)	
Month 3		N	12	14	12	14	12	12
		Mean	6.97	79.47	83.32	79.47	83.32	83.32
		SD	2.720	23.459	18.333	23.459	18.333	18.333
		Median	6.5	81.6	88.9	81.6	88.9	88.9
		Minimum	3.3	8.9	29.2	8.9	29.2	29.2
Month 4		Maximum	11.4	104.5	96.3	104.5	96.3	96.3
		N	10	14	11	14	11	11
		Mean	8.38	77.81	95.51	77.81	95.51	95.51
		SD	3.400	25.375	15.794	25.375	15.794	15.794
		Median	7.1	76.0	93.6	76.0	93.6	93.6
Month 5		Minimum	4.9	7.9	78.3	7.9	78.3	78.3
		Maximum	16.9	117.4	139.0	117.4	139.0	139.0
Month 5	Pre-infusion	N	9	13	11	13	11	11
		Mean	9.48	70.88	91.94	70.88	91.94	91.94
		SD	2.606	26.535	10.720	26.535	10.720	10.720
		Median	9.1	75.1	94.8	75.1	94.8	94.8
		Minimum	6.1	9.0	75.4	9.0	75.4	75.4
Month 5	2-Hr Post infusion	Maximum	15.6	115.0	106.9	115.0	106.9	106.9
		N	9	11	10	11	10	10
		Mean	11.03	92.09	99.19	92.09	99.19	99.19
		SD	6.203	8.267	10.200	8.267	10.200	10.200
		Median	7.6	95.0	97.4	95.0	97.4	97.4
Month 5		Minimum	4.9	79.7	82.5	79.7	82.5	82.5
		Maximum	21.7	103.4	114.6	103.4	114.6	114.6

FIG. 5C

Levels of receptor saturation maintained in the MS study					
Visit	Sampling Time	Placebo (n=71)		3.0 mg/kg Antegren (n=68)	
		Statistic		Statistic	
Month 5	24-Hr Post infusion	N		N	
		8		12	
		Mean		92.04	
		SD		15.256	
		Median		94.6	
		Minimum		49.5	
		Maximum		106.4	
Week 21		N		12	
		Mean		80.25	
		SD		24.078	
		Median		86.2	
		Minimum		5.1	
		Maximum		94.6	
		N		11	
		Mean		92.27	
		SD		11.557	
		Median		88.9	
		Minimum		77.3	
		Maximum		112.9	
		N		10	
		Mean		89.08	
		SD		32.364	
		Median		95.9	
		Minimum		4.8	
		Maximum		123.2	
		N		10	
		Mean		90.44	
		SD		18.174	
		Median		81.6	
		Minimum		73.5	
		Maximum		127.8	
		N		10	
		Mean		97.24	
		SD		23.374	
		Median		90.9	
		Minimum		78.8	
		Maximum		154.8	

FIG. 5D

Levels of receptor saturation maintained in the MS study

Visit	Sampling Time	Statistic	Placebo (n=71)	3.0 mg/kg Antegren (n=68)	6.0 mg/kg Antegren (n=74)
Month 6		N	10	13	9
		Mean	8.65	67.32	80.59
		SD	3.574	29.838	27.738
		Median	8.2	80.2	85.4
		Minimum	4.4	6.4	10.0
		Maximum	16.7	102.5	106.2
Month 7		N	10	12	8
		Mean	7.69	42.10	80.46
		SD	1.281	24.011	27.664
		Median	7.7	41.1	67.2
		Minimum	5.1	4.7	62.5
		Maximum	10.4	88.0	145.2
Visit	Sampling Time	Statistic	Placebo (n=71)	3.0 mg/kg Antegren (n=68)	6.0 mg/kg Antegren (n=74)
Month 8		N	10	13	8
		Mean	7.60	14.09	34.16
		SD	1.726	10.214	21.619
		Median	6.9	12.1	29.6
		Minimum	6.0	6.2	11.8
		Maximum	10.7	45.0	59.6
Month 9		N	5	7	7
		Mean	9.52	5.88	9.45
		SD	2.701	2.246	4.644
		Median	9.0	5.6	7.7
		Minimum	6.3	3.7	5.5
		Maximum	13.5	10.4	18.0

FIG. 5E

Levels of receptor saturation maintained in the MS study

Visit	Sampling Time	Statistic	Placebo (n=71)	3.0 mg/kg Antegren (n=68)	6.0 mg/kg Antegren (n=74)
Month 10		N	2	3	4
		Mean	7.78	8.65	9.17
		SD	2.029	0.635	1.655
		Median	7.8	8.6	9.3
		Minimum	6.3	8.0	7.1
Month 12		Maximum	9.2	9.3	11.0
		N	1	1	1
		Mean	8.80	6.04	6.61
		SD			
		Median	8.8	6.0	6.6
Early Termination		Minimum	8.8	6.0	6.6
		Maximum	8.8	6.0	6.6
		N	0	1	1
		Mean		94.51	32.29
		SD			
Early Termination		Median		94.5	32.3
		Minimum		94.5	32.3
		Maximum		94.5	32.3
		N			
		Mean			
		SD			

FIG. 5F

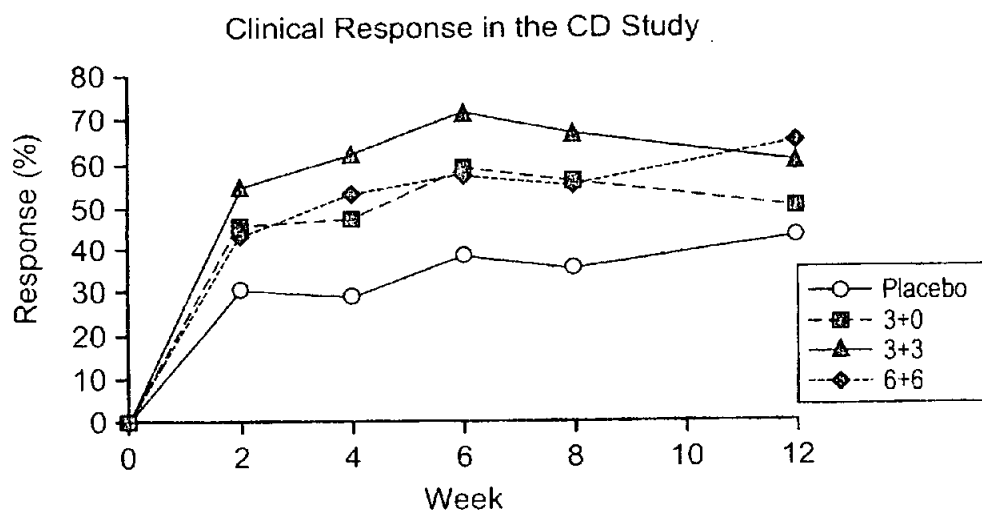


FIG. 6

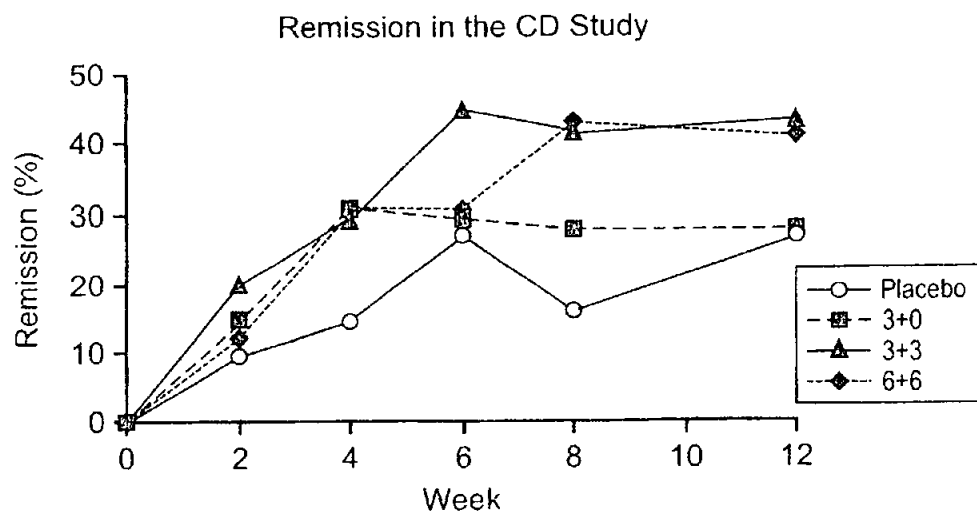


FIG. 7

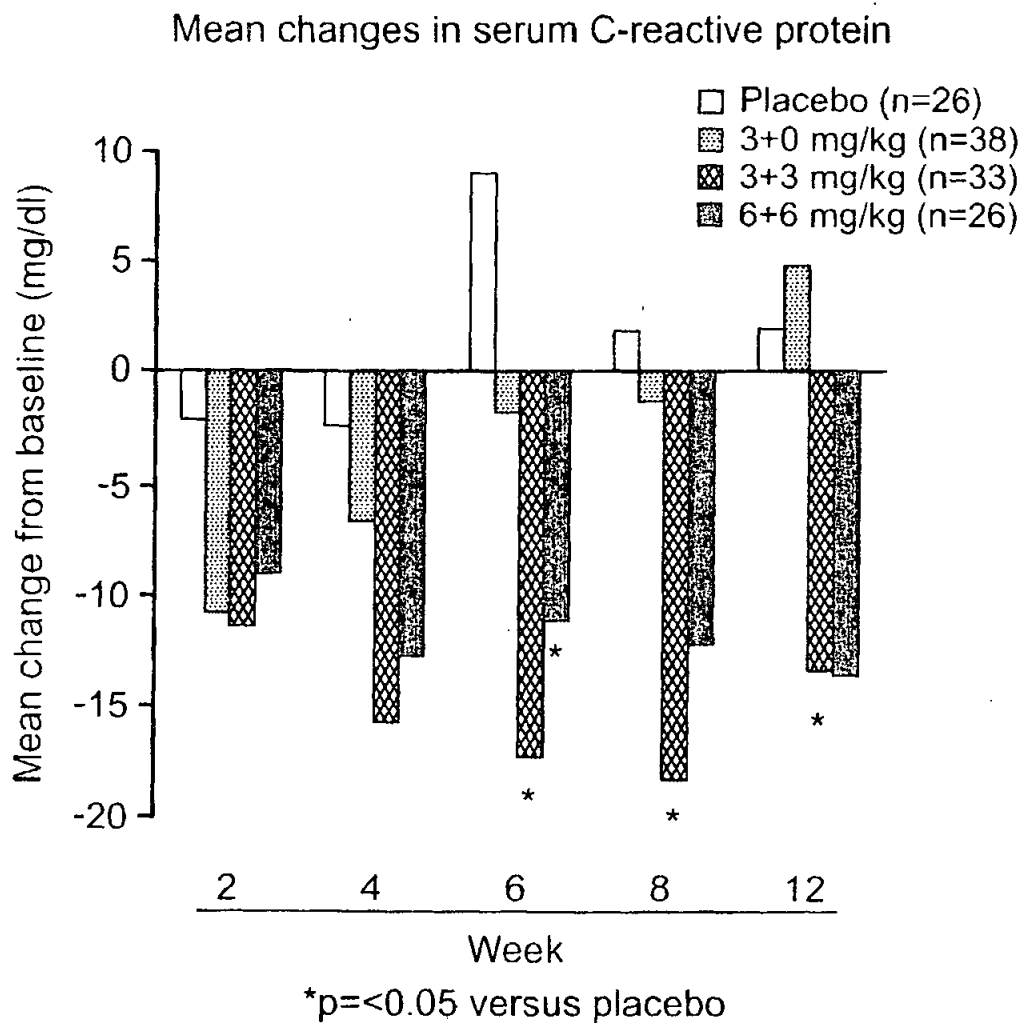


FIG. 8

The effect of natalizumab on circulating eosinophils in patients with active Crohn's disease and ulcerative colitis.

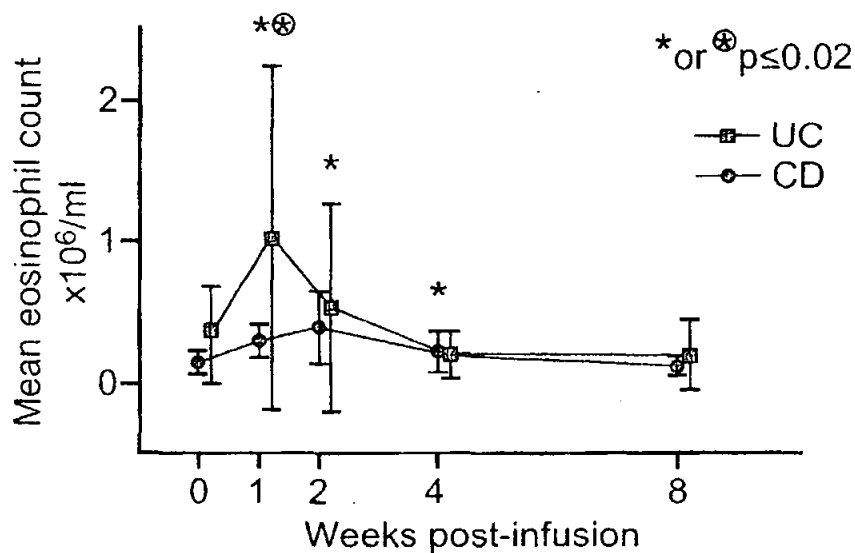


FIG. 9A

The effect of natalizumab on circulating monocytes in patients with active Crohn's disease and ulcerative colitis.

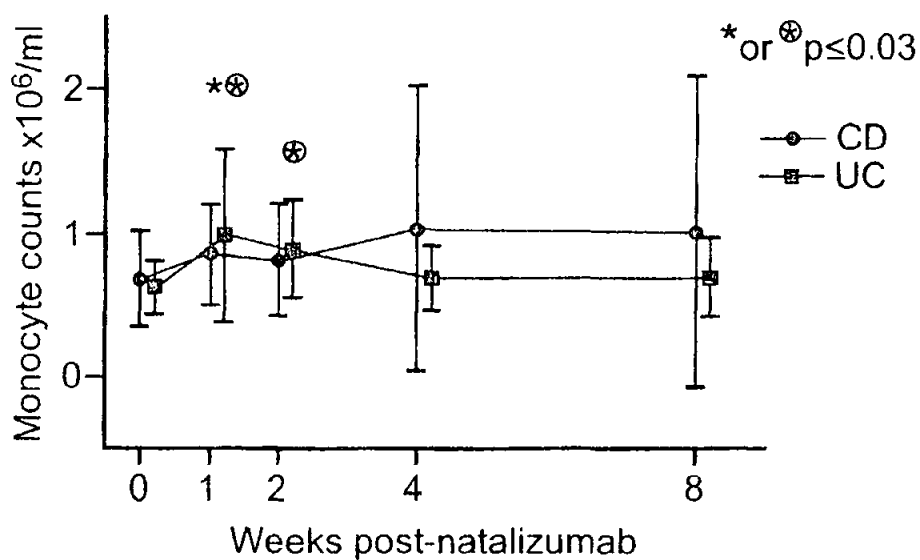


FIG. 9B

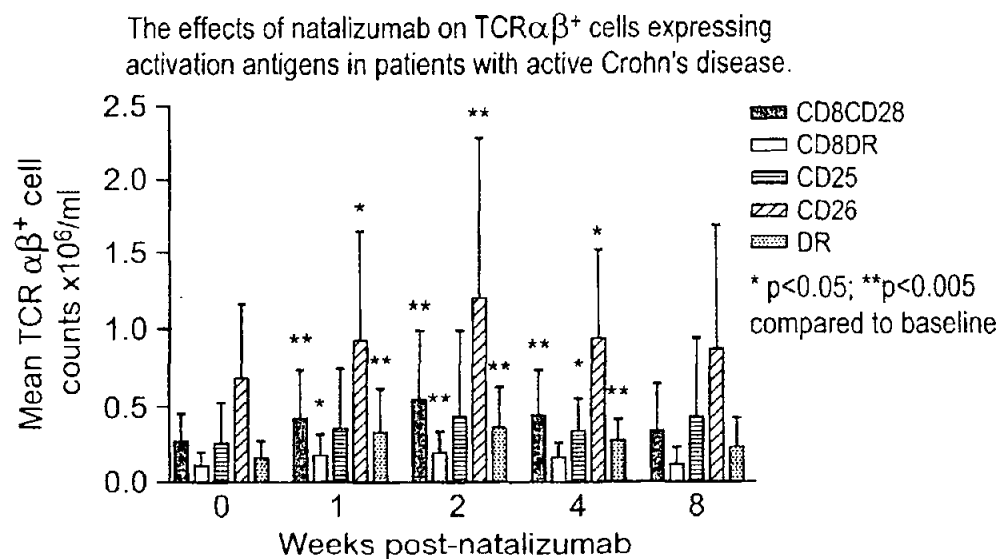


FIG. 10A

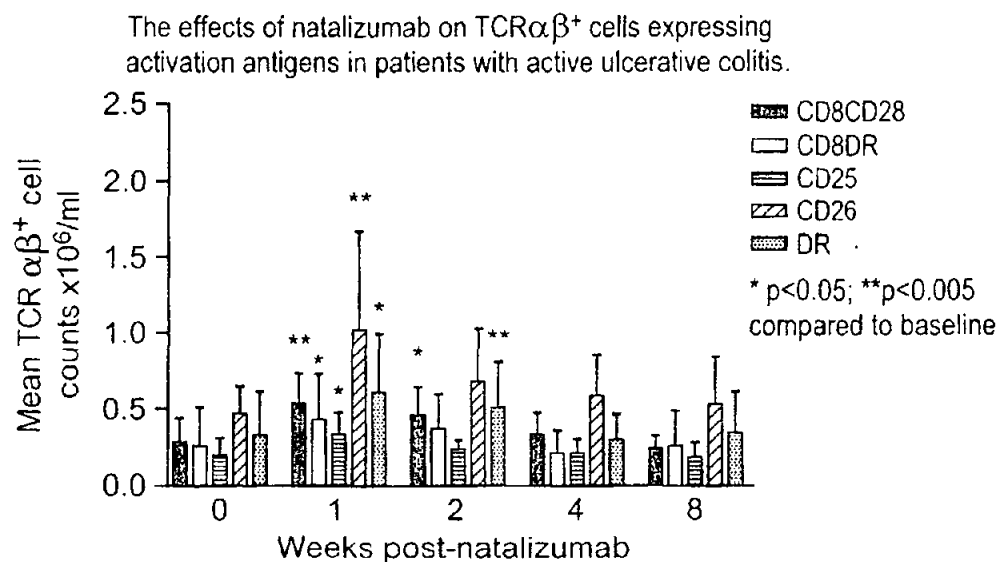


FIG. 10B

The effects of natalizumab on TCR $\alpha\beta$ ⁺ cells expressing activation antigens and memory and naive markers in patients with active Crohn's disease.

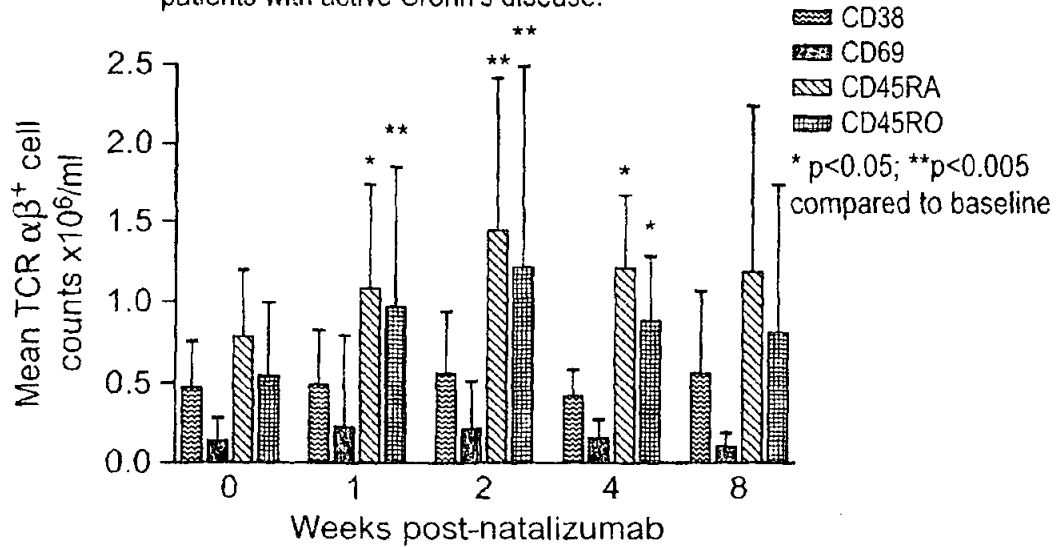


FIG. 10C

The effects of natalizumab on TCR $\alpha\beta$ ⁺ cells expressing activation antigens, memory and naive markers in patients with active ulcerative colitis.

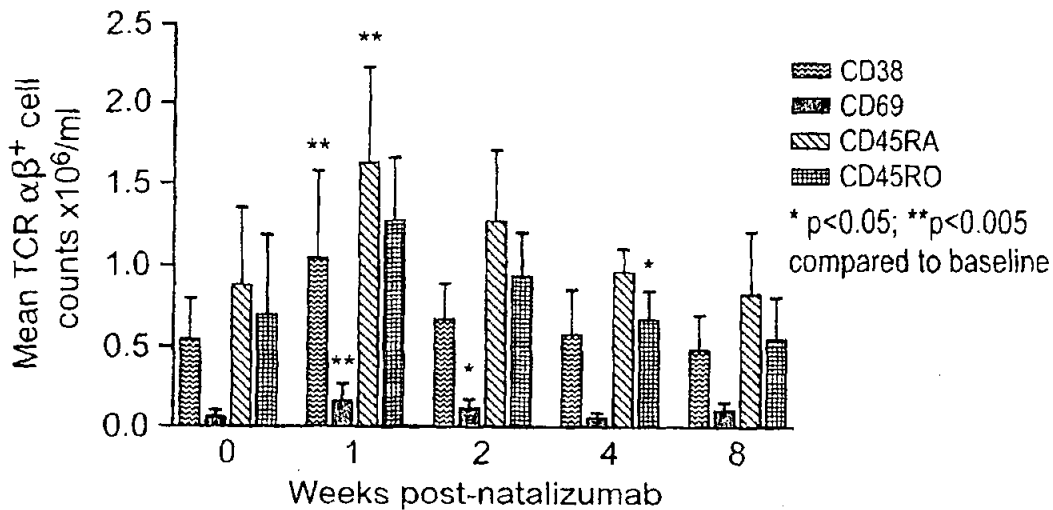


FIG. 10D

The effects of natalizumab on circulating $\text{TCR}\gamma\delta^+$ and NK-type cells in patients with active Crohn's disease.

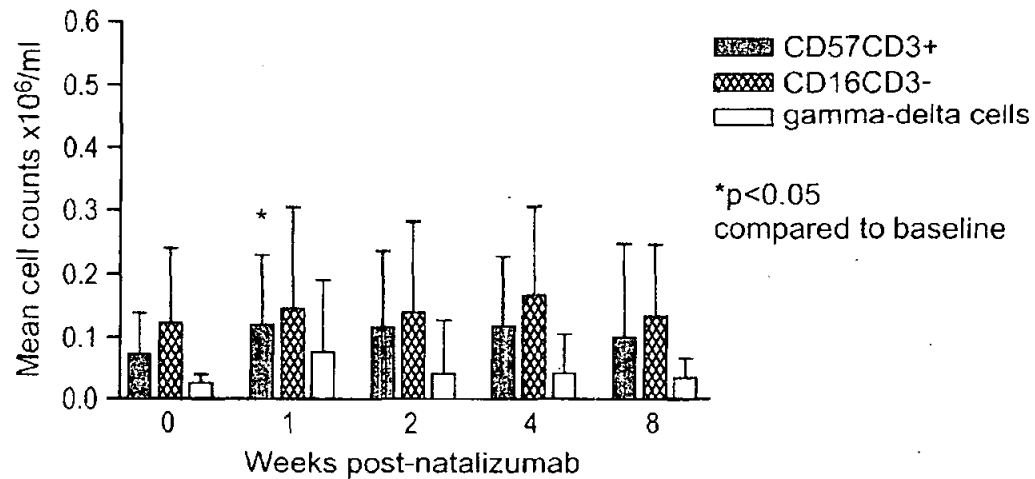


FIG. 11A

The effects of natalizumab on circulating $\text{TCR}\gamma\delta^+$ and NK-type cells in patients with ulcerative colitis.

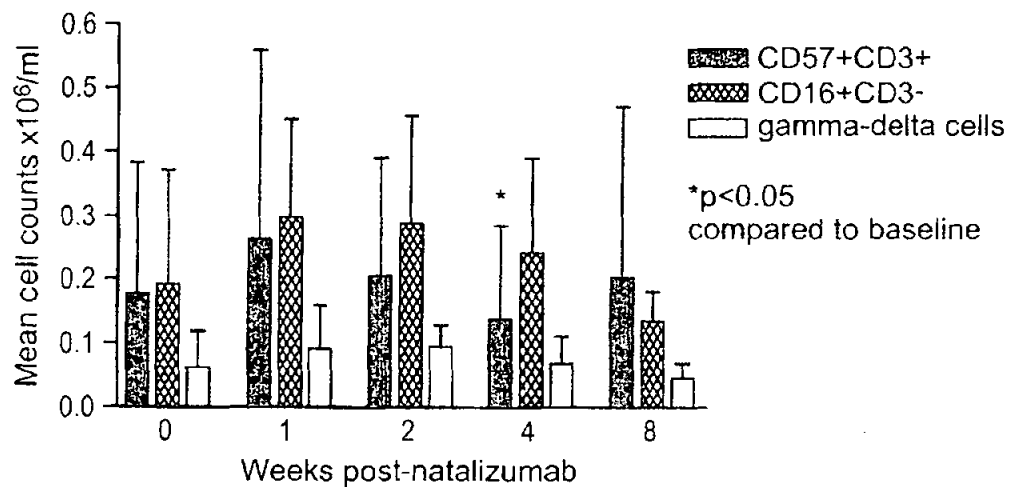


FIG. 11B

ADMINISTRATION OF AGENTS FOR THE TREATMENT OF INFLAMMATION

[0001] This application claims priority under 35 U.S.C. §119 to U.S. Provisional Application Nos. 60/374,501 entitled ADMINISTRATION OF AGENTS FOR THE TREATMENT OF INFLAMMATION and filed on Apr. 23, 2002, and Provisional Application No. 60/360,134, entitled ADMINISTRATION OF AGENTS FOR THE TREATMENT OF INFLAMMATION and filed on Feb. 25, 2002 the entire content of which are hereby incorporated by reference in their entirety for all purposes.

FIELD OF THE INVENTION

[0002] This invention relates generally to agents that specifically bind to and inhibit an integrin receptor comprising an alpha-4 ($\alpha 4$) subunit, and therapeutic uses of the same.

BACKGROUND OF THE INVENTION

[0003] Inflammation is a response of vascularized tissues to infection or injury and is affected by adhesion of leukocytes to the endothelial cells of blood vessels and their infiltration into the surrounding tissues. In normal inflammation, the infiltrating leukocytes release toxic mediators to kill invading organisms, phagocytize debris and dead cells, and play a role in tissue repair and the immune response. However, in pathological inflammation, infiltrating leukocytes are over-responsive and can cause serious or fatal damage. See, e.g., Hickey, *Psychoneuroimmunology II* (Academic Press 1990).

[0004] The integrins are a family of cell-surface glycoproteins involved in cell-adhesion, immune cell migration and activation. Alpha-4 integrin is expressed by all circulating leukocytes except neutrophils, and forms heterodimeric receptors in conjunction with either the beta1 or beta7 integrin subunits; both alpha-4 beta-1 ($\alpha 4 \beta 1$) and alpha-4 beta-7 ($\alpha 4 \beta 7$) dimers play a role in the migration of leukocytes across the vascular endothelium (Springer et al., 1994 *Cell* 76: 301-14; and Butcher et al., 1996 *Science* 272: 60-6) and contribute to cell activation and survival within the parenchyma (Damle et al., 1993 *J. Immunol.* 151: 2368-79; Koopman et al., 1994 *J. Immunol.* 152: 3760-7; and Leussink et al., 2002 *Acta Neuropathol.* 103:131-136).

[0005] Specifically, alpha-4 beta-1 (also known as very late antigen-4 [VLA-4]), binds to vascular cell adhesion molecule-1 (VCAM-1) (Lobb et al., 1994 *J. Clin. Invest.* 94:1722-8), which is expressed by the vascular endothelium at many sites of chronic inflammation (Bevilacqua et al., 1993 *Annu. Rev. Immunol.* 11: 767-804; and Postigo et al., 1993 *Res. Immunol.* 144:723-35). The alpha-4 beta-7 dimer interacts with mucosal addressin cell adhesion molecule (MAdCAM-1), and mediates homing of lymphocytes to the gut (Farstad et al., 1997 *Am. J. Pathol.* 150: 187-99; and Issekutz et al., 1991 *J. Immunol.* 147: 4178-84). Expression of MAdCAM-1 on the vascular endothelium is also increased at sites of inflammation in the intestinal tract of patients with inflammatory bowel disease (IBD) (Briskin et al., 1997 *Am. J. Pathol.* 151: 97-110).

[0006] Adhesion molecules such as alpha-4 integrins are potential targets for therapeutic agents. For instance, the VLA-4 receptor, of which alpha-4 integrin is a subunit, is an important target because of its interaction with a ligand

residing on brain endothelial cells. Diseases and conditions resulting from brain inflammation have particularly severe consequences. In another example, the alpha-4 beta-7 integrin dimer is an important target due to its involvement in lymphocyte homing and pathological inflammation in the gastrointestinal tract.

[0007] Alpha-4 beta-1 integrin is expressed on the extracellular surface of activated lymphocytes and monocytes, which have been implicated in the pathogenesis of acute inflammatory brain lesions and blood brain barrier (BBB) breakdown associated with multiple sclerosis (MS) (Coles et al., 1999 *Ann. Neurol.* 46(3): 296-304). Agents against alpha-4 integrin have been tested for their anti-inflammatory potential both in vitro and in vivo in animal models. See Yednock et al., 1992 *Nature* 356: 63-66; U.S. Pat. No. 5,840,299 issued to Bendig, et al. on Nov. 24, 1998, and U.S. Pat. No. 6,001,809 issued to Thorsett et al. on Dec. 14, 1999. The in vitro experiments demonstrate that alpha-4 integrin antibodies block attachment of lymphocytes to brain endothelial cells. Experiments testing the effect of alpha-4 integrin antibodies on animals having an artificially induced condition simulating multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), have demonstrated that administration of anti-alpha-4 integrin antibodies prevents inflammation of the brain and subsequent paralysis in the animals. Collectively, these experiments identify anti-alpha-4 integrin antibodies as potentially useful therapeutic agents for treating multiple sclerosis and other inflammatory diseases and disorders.

[0008] In another specific example of pathological inflammation involving alpha-4 integrins, Crohn's disease (CD) is a chronic, incurable, relapsing, transmural inflammation of the intestinal tract. The disease is characterized by inappropriate immune cell migration and activation in the intestinal mucosa involving T cells, macrophages and neutrophils (Schreiber et al., 1991 *Gastroenterology* 101: 1020-30). First-line medical therapies for Crohn's disease include 5-aminosalicylates (5-ASAs), which have low efficacy, and corticosteroids, which have various short- and long-term side effects (Munkholm et al., 1994 *Gut* 35: 360-2). Patients refractory to first-line therapies are treated with immunosuppressive agents such as azathioprine, 6-mercaptopurine, and methotrexate, but these agents have a slow onset of action and potentially serious side effects (Stein et al., 2001 *Surg. Clin. North Am.* 81: 71-101, viii). More recently, biologic agents with a faster onset of action have been introduced for use in treating Crohn's disease, but such agents are similarly plagued by issues such as long-term efficacy and side effects.

SUMMARY OF THE INVENTION

[0009] The present invention provides methods of chronically reducing pathological inflammation in a patient via the chronic administration of an agent that selectively binds to alpha-4 integrin. The chronic dosage regime of an alpha-4 agent is designed such that (1) the agent specifically binds to alpha-4 integrin or an integrin, dimer comprising alpha-4 integrin, and (2) the agent is administered repeatedly to maintain alpha-4 integrin receptor saturation at a sufficient level to suppress pathological inflammation. The agent of the invention can be useful in the suppression of inflammation via binding and inhibition of all integrin dimers comprising the alpha-4 subunit, or it can be designed to bind to a specific dimer, e.g., alpha-4 beta-1.

[0010] In one embodiment, the efficacy of a chronic dosage regime can be determined by the measurement of saturation of a specific integrin dimer. In a particular example, it is believed that alpha-4 beta-1 integrin dimer is involved in multiple sclerosis, and thus the level of saturation required for an efficacious chronic administration regime can be measured via measurement of the saturation of the alpha-4 beta-1 dimer receptor.

[0011] In another embodiment, where multiple integrin dimers are believed to be involved in a pathological inflammation, saturation of a combination of dimer receptors can be measured to determine the efficacy of a chronic administration regime. In a specific example, both the alpha-4 beta-1 and the alpha-4 beta-7 dimers are believed to have involvement in pathological inflammation associated with inflammatory bowel disease. Thus, measurement of both alpha-4 beta-1 and alpha-4 beta-7 saturation levels may be beneficial in determining the efficacy of a particular chronic administration regime.

[0012] In one embodiment, the success of a chronic dosage regime can be determined by assessing a physiological marker of the pathological inflammation. For example, in a chronic dosage regime administered for MS, the success of the dosage regime can be confirmed by detection of the levels of brain lesions using an imaging technique, e.g., magnetic resonance imaging (MRI). In another example, in a chronic dosage regime administered for CD, the success of the dosage regime can be confirmed by detection of the levels of serum C-reactive protein levels in a patient. In yet another example, the success of the dosage regime can be determined using a set group of criteria associated with wellness, e.g., reduction in CDAI in a CD patient.

[0013] Another embodiment of the invention contemplates the treatment of an inflammatory disease of the gastrointestinal tract (e.g., Crohn's disease, ulcerative colitis and inflammatory bowel disease) of a subject comprising administering a therapeutically effective amount of a composition comprising natalizumab sufficient to treat or ameliorate said inflammatory disease of the gastrointestinal tract in said subject.

[0014] In a specific embodiment, the invention features a dosage regime wherein repeated administration of an agent is provided to provide a level of alpha-4 integrin receptor saturation of 65-100% in a patient, thereby providing chronic suppression of pathological inflammation in the patient. In another specific embodiment, the agent is repeatedly administered to provide levels of at least about 75-100% in a patient. In yet another specific embodiment, the agent is repeatedly administered to provide levels of at least about 80-100% in a patient.

[0015] A feature of the invention is that undesirable effects of pathological inflammation can be suppressed long-term in a patient, e.g., over a period of six months, one year, two years, or more.

[0016] An aspect of the method of the invention is that sufficient levels of an anti-alpha-4 integrin agent are maintained over long periods to suppress pathological inflammation over those periods.

[0017] A feature of the invention is that the dosage form provides lower levels of pathological inflammation over longer periods as compared to a single dose.

[0018] An advantage of the invention is that agents used in the methods of the invention are well tolerated and have low toxicity.

[0019] These and other objects, advantages, and features of the invention will become apparent to those persons skilled in the art upon reading the details of the methods and formulations as more fully described below.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0020] FIG. 1 is a line graph illustrating the cumulative mean number of new Gd-enhancing lesions in MS patients following dosing with natalizumab.

[0021] FIG. 2 shows the percentage of active scans at each time point during the natalizumab MS study. Active scans are those containing one or more new Gd-enhancing lesions.

[0022] FIGS. 3A-C and 4A-C show the serum concentrations of natalizumab following the dosage regime time points in an MS study. FIGS. 3A-C show the levels for the 3-mg/kg study; FIGS. 4A-C show the levels for the 6-mg/kg study.

[0023] FIGS. 5A-F illustrates the levels of receptor saturation maintained in the MS study. Levels shown are percentage values.

[0024] FIGS. 6 and 7 show the percentage of Patients Achieving Predefined Criteria of Clinical Response (FIG. 6) or Remission (FIG. 7) following dosing in the CD study.

[0025] FIG. 8 illustrates mean changes in serum C-reactive protein in a subset of patients who had elevated C-reactive protein at base-line in the CD study. Base-line values were (in mg/l): Placebo, 38.44 (N=26); 3+0 mg/kg Group, 32.35 (N=38); 3+3 mg/kg Group, 41.16 (N=33); and 6+6 mg/kg Group, 333 (N=26).

[0026] FIGS. 9A-B demonstrate respectively the effects of natalizumab on circulating eosinophils in patients with active Crohn's disease (CD) and ulcerative colitis (UC). FIG. 9A demonstrates that natalizumab significantly increased circulating eosinophil counts in Crohn's disease (n=18) and ulcerative colitis patients (n=12) after 3 mg/kg natalizumab infusion. FIG. 9B shows that administration of natalizumab significantly increased monocyte counts in active Crohn's disease and ulcerative colitis patients after 3 mg/kg natalizumab infusion.

[0027] FIGS. 10A-D demonstrate the impact of natalizumab administration on TCR $\alpha\beta^+$ cells expressing activation antigen. Panel A demonstrates the effect of natalizumab in patients with active Crohn's disease, i.e., a significant increase of TCR $\alpha\beta^+$ cells expressing CD26, HLA-DR, CD8CR and CD8 CD28 to at least four weeks post 3 mg/kg natalizumab infusion. Panel B demonstrates the effects of natalizumab on TCR $\alpha\beta^+$ cells expressing activation antigens in patients with activated ulcerative colitis, i.e., significant increase in TCR $\alpha\beta^+$ cells expressing CD26, HLA-DR, CD8DR and CD8CD28 to at least four weeks post 3 mg/kg natalizumab infusion. Panel C demonstrates the effects of natalizumab administration on TCR $\alpha\beta^+$ cells expressing activation antigens and memory and naive markers in patients with active Crohn's disease, i.e., significant increase of memory (CD45RO) and naive (CD45RA) TCR $\alpha\beta^+$ cells to at least four weeks post-natalizumab infusion. Panel D shows the effects of natalizumab on TCR $\alpha\beta^+$ cells express-

ing activation antigens, memory and naive markers in patients with active ulcerative colitis, i.e., significant increase of memory (CD45RO), naive (CD45RA), CD69 and CD38 TCR $\alpha\beta$ ⁺ cells at one week post natalizumab administration.

[0028] FIGS. 11A-B demonstrate the effect of natalizumab on circulating TCR $\alpha\beta$ ⁺ and NK-type cells in patients with active Crohn's disease and with ulcerative colitis respectively.

DETAILED DESCRIPTION OF THE INVENTION

[0029] Before the present methods and therapeutic agents are described, it is to be understood that this invention is not limited to particular methods and therapeutic agents described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0030] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0031] It must be noted that as used herein, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an antibody" includes a plurality of such antibodies and reference to "the dosage" includes reference to one or more dosages and equivalents thereof known to those skilled in the art, and so forth.

[0032] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

Definitions

[0033] The term "anti-alpha-4 agent" as used herein refers to any agent, which binds specifically to an integrin comprising an alpha-4 subunit and inhibits activity of the integrin.

[0034] This includes agents that specifically bind to alpha-4 integrin, as well as agents that bind to an integrin dimer that comprises the alpha-4 integrin, e.g., alpha-4 beta-1 ($\alpha 4\beta 1$) or alpha-4 beta-7 ($\alpha 4\beta 7$). The term "agents" is meant to include synthetic and recombinant molecules (e.g., antibodies, small molecules, peptides, or other synthetically produced molecules or compounds, as well as recombinantly produced gene products) as well as naturally-occurring compounds (e.g., polypeptides, antibodies, and the like).

[0035] The term "efficacy" as used herein in the context of a chronic dosage regime refers to the effectiveness of a particular treatment regime. Efficacy can be measured based on change the course of the disease in response to an agent of the present invention. For example, in the treatment of MS, efficacy can be measured by the frequency of relapses in relapsing-remitting MS, and by the presence or absence of new lesions in the central nervous system as detected using methods such as MRI.

[0036] The term "success" as used herein in the context of chronic treatment regimes refers to the effectiveness of a particular treatment regime. This includes a balance of efficacy, toxicity (e.g., side effects and patient tolerance of a formulation or dosage unit), patient compliance, and the like. For a chronic administration regime to be considered "successful" it must balance different aspects of patient care and efficacy to produce the most favorable patient outcome.

[0037] The terms "specifically binds" or "binds specifically" as used herein refer to the situation in which one member of a specific binding pair will not show any significant binding to molecules other than its specific binding partner (e.g., an affinity of about 1,000x or more for its binding partner). In the present invention, the anti-alpha-4 integrin agent will not show significant binding to any polypeptide other than an alpha-4 integrin or a receptor comprising an alpha-4 integrin. For example, antibodies used in the methods of the invention that bind to an alpha-4 integrin with a binding affinity of 10^7 mole/l or more, preferably 10^8 mole/liters or more, are said to bind specifically to an alpha-4 integrin.

[0038] The term "substantially homologous" as used herein is intended to mean any polypeptide that has an alteration in the sequence such that a functionally equivalent amino acid is substituted for one or more amino acids in the polypeptide, thus producing a change that has no or relatively little effect on the binding properties of the polypeptide. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity.

[0039] The terms "elicits an immune response" and "elicits a host immune response" as used herein refer to the production of an immunological response to a receptor comprising an alpha-4 integrin in a subject upon introduction of an agent of the invention to the subject. An immune response in the subject can be characterized by serum reactivity with an alpha-4 integrin receptor that is at least twice that of an untreated subject, more preferably three times the reactivity of an untreated subject, and even more preferably at least four times the reactivity of an untreated subject, with serum immunoreactivity measured using a serum dilution of approximately 1:100.

[0040] The term "excipient material" is intended to mean any compound forming a part of the formulation that is intended to act merely as a carrier, i.e. not intended to have biological activity itself.

[0041] The term "adjuvant" as used herein refers to a composition additive that augments the immune response to an agent of the invention but which will not on its own elicit an immune response. Adjuvants may augment the immune response using a variety of biological mechanisms, including but not limited to lymphocytic recruitment, T cell stimulation, B cell stimulation, and macrophage stimulation.

[0042] The terms "treating", and "treatment" and the like are used herein to generally mean obtaining a desired pharmacological and physiological effect. The effect may be prophylactic in terms of preventing or partially preventing a disease, symptom or condition thereof and/or may be therapeutic in terms of a partial or complete cure of a disease, condition, symptom or adverse effect attributed to the disease. The term "treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease and/or its symptoms or conditions. The invention is directed towards treating a patient's suffering from disease related to pathological inflammation. The present invention is involved in preventing, inhibiting, or relieving adverse effects attributed to pathological inflammation over long periods of time and/or are such caused by the physiological responses to inappropriate inflammation present in a biological system over long periods of time.

[0043] The term "pathological inflammation" as used herein refers to an inappropriate and chronic inflammation associated with disorders including, but not limited to, asthma, atherosclerosis, AIDS dementia, diabetes, inflammatory bowel disease, rheumatoid arthritis, transplant rejection, graft versus host disease, multiple sclerosis (especially to inhibit further demyelination), tumor metastasis, nephritis, atopic dermatitis, psoriasis, myocardial ischemia, chronic prostatitis, complications from sickle cell anemia, lupus erythematosus, and acute leukocyte mediated lung injury. Such inflammation is characterized by a heightened response of inflammatory cells, including infiltrating leukocytes. Over time, such pathological inflammation often results in damage to tissue in the region of inappropriate inflammation.

[0044] By "Antegren®" is meant to include the antibody also known as AN100226 (antibody code number) or natalizumab (USAN name). Antegren is a recombinant, humanized anti-alpha-4 integrin antibody. Preferably the disease or condition being treated in the mammal is one which is modulated when a therapeutically effective dose of Antegren® is administered.

General Aspects of the Invention

[0045] The present invention is based on the surprising result that chronic administration of an emerging class of new compounds known as selective adhesion molecule inhibitors (SAMIs) is sufficient to provide the maintenance of chronic suppression of inflammation in disorders involv-

ing integrin dimers. Upon cessation of the repeated dosage regime, the suppression of the inflammation is reversed (see, e.g., FIG. 2). Previous treatments of inflammatory inhibitors have approached the dosage regimes quite differently, in the belief that the administration of an inflammatory inhibitor would cause a reaction of the body's own response system, which would in turn lead to a recognition of the inflammation as pathological and a resulting chronic relief of the pathological inflammation. What the inventors have shown herein is that a chronic dosage regime is not only more effective than a short-term dosage regime, but in fact it is required to maintain the suppression of pathological inflammation. Thus, in order to realize some of the more important advantages of the invention, the levels of an anti-alpha-4 integrin agent need to be maintained over a number of months or even years.

[0046] The present invention is based on the results of a large, randomized, placebo-controlled trial of an anti-alpha-4 integrin antibody, natalizumab, in patients with relapsing MS or with moderate to severely active CD. Natalizumab is a recombinant, humanized, monoclonal antibody antagonist against alpha-4 integrin. Results from these two trials have shown that treatment with natalizumab improved the signs and symptoms of patients with MS and CD. The invention is also intended to include other chimeric antibodies, including primatized™ antibodies.

[0047] In a general sense the method of the invention does not involve any particular mode of administration, since the mode of administration is dependent upon the form of the active agent and the formulation developed to administer the active agent(s). However, the specific examples described here were obtained using parenteral administration of natalizumab. Although the present invention is described using an antibody that specifically binds to alpha-4 integrin, it is also intended to include chronic administration of, for example, bivalent or multivalent antibodies that recognize both partners of an integrin dimer, provided the dimer comprises an alpha-4 integrin.

[0048] The general concept of the invention relates to introducing relatively constant amounts of an active agent to a patient's circulatory system over a period of months or years. This chronic introduction of an agent that selectively binds to alpha-4 integrin or a dimer comprising alpha-4 integrin results in suppression of pathological inflammation being maintained at a constant level over a period of time. By maintaining therapeutic levels of an active agent for a period of time, pathological inflammation can be chronically suppressed in the patient.

[0049] In a very specific sense, the invention involves obtaining and maintaining a receptor saturation level in a human patient of a dimer comprising alpha-4 integrin in a range of from about 65% to about 100%, more preferably between about 75% to about 100%, and even more preferably between about 80% to about 100%. These receptor saturation levels are maintained at these levels chronically (e.g., over a period of 6 months or so) to allow for continued suppression of pathological inflammation. Agents that Selectively Bind to Alpha-4 Integrins

[0050] Various types of agents with the ability to bind to and inhibit alpha-4 integrin activity can be used in the practice of the invention. Many such agents have been identified and characterized, and specific agents are

described below. Given the teachings disclosed herein, it is well within the skill of one in the art to identify other agents that will be able to inhibit the alpha-4-comprising integrin dimers in a manner that biologically mimics or is similar to the specifically described agents, and the present invention is intended to include the chronic administration of such agents and combinations of such agents.

[0051] Antibodies

[0052] In one specific embodiment, the agents of the invention are antibodies or immunologically active fragments thereof that selectively bind to an alpha-4 integrin or a dimer comprising alpha-4, such as alpha-4 beta-1 or alpha-4 beta-7.

[0053] When the agent of the invention is an antibody, a monoclonal antibody is preferred. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different epitopes, each monoclonal antibody is directed against a single epitope on the antigen. A second advantage of monoclonal antibodies is that they are synthesized by means that are uncontaminated by other immunoglobulins, e.g., by phage display or isolation from a hybridoma. Although the present invention intends to encompass both polyclonal and monoclonal antibodies as agents of the invention, monoclonal antibodies are preferred as they are highly specific, and the invention is thus discussed primarily in terms of monoclonal antibodies.

[0054] In addition, other antibodies can be identified using techniques available in the art. For example, monoclonal antibodies of the present invention can be produced using phage display technology. Antibody fragments that selectively bind to an alpha-4 integrin or a dimer comprising an alpha-4 integrin are then isolated. Exemplary preferred methods for producing such antibodies via phage display are disclosed in U.S. Pat. Nos. 6,225,447; 6,180,336; 6,172,197; 6,140,471; 5,969,108; 5,885,793; 5,872,215; 5,871,907; 5,858,657; 5,837,242; 5,733,743; and 5,565,332.

[0055] Monoclonal antibodies can also be produced using the conventional hybridoma methods. These methods have been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens, and can also be used to produce monoclonal antibodies of the present invention. For example, mice (e.g., Balb/c mice) can be immunized with an antigenic alpha-4 integrin epitope by intraperitoneal injection. After sufficient time has passed to allow for an immune response, the mice are sacrificed, and the spleen cells obtained and fused with myeloma cells, using techniques well known in the art. The resulting fused cells, hybridomas, are then grown in a selective medium, and the surviving cells grown in such medium using limiting dilution conditions. After cloning and recloning, hybridomas can be isolated that secrete antibodies (for example, of the IgG or IgM class or IgG1 subclass) that selectively bind to the target, alpha-4 integrin or a dimer comprising an alpha-4 integrin. To produce agents specific for human use, the isolated monoclonal can then be used to produce chimeric and humanized antibodies.

[0056] Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies (e.g., scFv), Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id)

antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments that can comprise any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep, monkey, rabbit, goat, guinea pig, pig, camel, horse, or chicken (or other avian). As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati et al.

[0057] Chimeric and humanized antibodies can be produced from non-human antibodies, and can have the same or similar binding affinity as the antibody from which they are produced. Techniques for producing chimeric antibodies (Morrison et al., 1984 *Proc. Natl. Acad. Sci.* 81: 6851; Neuberger et al., 1984 *Nature* 312: 604; Takeda et al., 1985 *Nature* 314: 452) include splicing the genes from, e.g., a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity; such antibodies are within the scope of this invention. For example, a nucleic acid encoding a variable (V) region of a mouse monoclonal antibody can be joined to a nucleic acid encoding a human constant (C) region, e.g., IgG1 or IgG4. The resulting antibody is thus a species hybrid, generally with the antigen binding domain from the non-human antibody and the C or effector domain from a human or primate antibody.

[0058] Humanized antibodies are antibodies with variable regions that are primarily from a human antibody (i.e., the acceptor antibody), but which have complementarity determining regions substantially from a non-human antibody (the donor antibody). See, e.g., Queen et al., *Proc. Nat'l. Acad. Sci. USA* 86: 10029-10033 (1989); WO 90/07861, U.S. Pat. Nos. 6,054,297; 5,693,761; 5,585,089; 5,530,101; and 5,224,539. The constant region or regions of these antibodies are generally also from a human antibody. The human variable domains are typically chosen from human antibodies having sequences displaying a high homology with the desired non-human variable region binding domains. The heavy and light chain variable residues can be derived from the same antibody, or a different human antibody. In addition, the sequences can be chosen as a consensus of several human antibodies, such as described in WO 92/22653.

[0059] A "primatizedTM antibody" is a recombinant antibody containing primate variable sequences or antigen binding portions, and human constant domain sequences. See Newman, *Biotechnology*, 1992, 10: 1455-60. Primatization of antibodies results in the generation of antibodies that

contain monkey variable domains and human constant sequences. For more details see U.S. Pat. No. 6,113,898. This technique modifies antibodies such that they are not rejected upon administration in humans because they are antigenic. This technique relies on immunization of cynomolgus monkeys with human antigens or receptors. This technique was developed to create high affinity monoclonal antibodies directed to human cell surface antigens.

[0060] Specific amino acids within the human variable region are selected for substitution based on the predicted conformation and antigen binding properties. This can be determined using techniques such as computer modeling, prediction of the behavior and binding properties of amino acids at certain locations within the variable region, and observation of effects of substitution. For example, when an amino acid differs between a non-human variable region and a human variable region, the human variable region can be altered to reflect the amino acid composition of the non-human variable region.

[0061] In a specific embodiment, the antibodies used in the chronic dosage regime of the present invention are humanized antibodies as disclosed in U.S. Pat. No. 5,840,299, which is incorporated herein by reference. In another embodiment, transgenic mice containing human antibody genes can be immunized with an antigenic alpha-4 integrin structure and hybridoma technology can be used to generate human antibodies that selectively bind to alpha-4 integrin.

[0062] Chimeric, human and/or humanized antibodies can be produced by using recombinant expression, e.g., expression in human hybridomas (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985)), in myeloma cells or in Chinese hamster ovary (CHO) cells. Alternatively, antibody coding sequences can be incorporated into transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal. See e.g., U.S. Pat. Nos. 6,197,946; 5,849,992; 5,565,362; 5,336,894; and 5,304,489. Suitable transgenes include transgenes having a promoter and/or enhancer from a mammary gland specific gene, for example casein or β -lactoglobulin.

[0063] Small Molecules

[0064] Small molecules for use in the present invention encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 4,000 Daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including, but not limited to: peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[0065] The small molecules can be obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic

compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs.

[0066] Anti-Alpha-4 Integrin Peptides

[0067] The methods of the invention can be performed with any peptide that is capable of binding to an alpha-4 integrin or a dimer comprising an alpha-4 subunit. Included in the methods of the invention are peptides that are substantially homologous to a region of the extracellular matrix or a natural ligand of the specific alpha-4 integrin receptor or receptors targeted. For example, for the chronic inhibition of alpha-4 beta-1 receptor, peptides can be used that comprise at least a portion of the fibronectin HICS region (e.g., peptides comprising at least a portion of the CS-1 peptide sequence or a sequence substantially homologous to the CS-1 sequence) can be used to bind to a receptor and inhibit the activity of the alpha-4 comprising integrin. See for example U.S. Ser. No. 08/452,098, which is incorporated by reference in its entirety.

[0068] Agents that Elicit an Immune Response

[0069] In a specific embodiment, the agents of the invention are peptides or peptidomimetics that comprise an immunogenic fragment of an alpha-4 integrin. An immunogenic fragment is any fragment that comprises an epitope of alpha-4, and generally has at least 3, 5, 7, 10, 15, 17 or 20 contiguous amino acids from a naturally occurring mammalian alpha-4 protein. The peptide sequence of both human and murine alpha-4 is accessible from GenBank (Accession Nos. AA59613 and NP_034706). One skilled in the art can readily design a peptide agent of the invention based on the amino acid sequence of alpha-4 or using the wild-type nucleotides that encode them (e.g., GenBank Accession Nos. L12002 and NM_01056, respectively). Once an appropriate peptide is designed, it can be initially screened against antibodies known to have the desired immunogenic activity, e.g., antibodies that selectively bind to an alpha-4 structure and are characterized by the ability to inhibit the activity of an integrin comprising alpha-4.

[0070] The immunogenic fragment may also be designed to have amino acid analogs or other structural elements that will enhance the immunogenic response. In particular, peptide fragments may have altered C- or N-terminal ends that enhance overall immunogenicity of the molecules while not impeding their ability to elicit an immune response. Examples of such analogs include, but are not limited to: alpha, alpha-disubstituted amino acids, N-alkyl amino acids, lactic acid, 4-hydroxyproline, gamma-carboxyglutamate, gamma-N,N,N-trimethyllysine, gamma-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, and 5-hydroxylysine. Other useful analogs can be found in Sigma, *Biochemicals and Reagents*, Sigma-Aldrich (2001). The fragment may also be detectably labeled to allow for tracking of the molecule within a subject following administration to a subject.

[0071] Peptides, analog structures, peptidomimetics and the like can be isolated from natural sources, and then optionally processed (e.g., via peptide cleavage) or alternatively synthesized by conventional techniques known in the art such as solid phase synthesis or recombinant expression. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press, New York, 2nd edition 1989). Automatic peptide synthesis can be performed using a commercially available apparatus from manufacturers such as Applied Biosystems (Foster City, Calif.), and methods of doing so are well established. Recombinant production of the proteins may be in prokaryotic, such as phage or bacterial cells or eukaryotic systems, such as yeast, insect, or mammalian cells. Alternatively, proteins can be produced using cell-free in vitro systems known in the art.

[0072] In another example, phage peptide display libraries can be used to express large numbers of peptides that can be screened in vitro to identify peptides that specifically bind to alpha-4 or a dimer comprising an alpha-4 integrin. Phage display technology provides a means for expressing a diverse population of random or selectively randomized peptides. Various methods of phage display and methods for producing diverse populations of peptides are well known in the art. For example, Ladner et al. (U.S. Pat. No. 5,223,409), describes methods for preparing diverse populations of binding domains on the surface of a phage. Ladner et al. describe phage vectors useful for producing a phage display library, as well as methods for selecting potential binding domains and producing randomly or selectively mutated

e.g., U.S. Pat. No. 5,264,563). These and other well known methods can be used to produce a phage display library, which can be subjected to the in vivo panning method of the invention in order to identify a peptide that homes to one or a few selected organs.

[0074] The molecules of a peptide phage display library also can be present as a conjugate, which can facilitate recovery or identification of the peptide of interest. As used herein, the term "conjugate" means a peptide or peptidomimetic of the library linked to a physical, chemical or biological moiety such as but not limited to a solid substrate, a plastic microbead, an oligonucleotide or a bacteriophage, and the like. The moiety can provide a means to identify or recover an agent.

[0075] Some agents used to elicit an immune response mimic the appropriate epitope for inducing an immune response against alpha-4 integrin but are too small to be immunogenic on their own. In this situation, a peptide agent can be linked to a suitable carrier to facilitate an immune response. Suitable carriers include serum albumins, keyhole limpet hemocyanin (KLH), immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, or a toxoid from other pathogenic bacteria, such as diphtheria, *E. coli*, cholera, or *H. pylori*, or an attenuated toxin derivative. Other carriers include T-cell epitopes that bind to multiple MHC alleles, e.g., at least 75% of all human MHC alleles. Such carriers are sometimes known in the art as "universal T-cell epitopes." Examples of universal T-cell epitopes include:

Influenza Hemagglutinin: HA ₃₀₇₋₃₁₉	PKYVKQNTLKLAT	(SEQ ID NO: 2)
PADRE	AKXVAAWTLKAAA,	(SEQ ID NO: 3)
	where X is preferably cyclohexylalanine, tyrosine, or phenylalanine	
Malaria CS: T3 epitope	EKKIAXMEKASSVFNV	(SEQ ID NO: 4)
Hepatitis B surface antigen: HBsAg ₁₉₋₂₈	FPLLTRLTI	(SEQ ID NO: 5)
Heat Shock Protein 65: hsp65 ₁₅₃₋₁₇₁	DQSIGDLIAEAMDKVGNeg	(SEQ ID NO: 6)
bacille Calmette-Guerin	QVHFQPLPPAVVKL	(SEQ ID NO: 7)
Tetanus toxoid: TT ₈₃₀₋₈₄₄	QYIKANSKFIGITEL	(SEQ ID NO: 8)
Tetanus toxoid: TT ₄₁₇₋₄₆₇	FNNFTVSFWLRVFKVSASHLE	(SEQ ID NO: 9)
HIV gp120 T1	KQIINMWQEVGKAMYA.	(SEQ ID NO: 10)

binding domains. Screening of a phage display library generally involves in vitro panning of the library using a purified target molecule. Phage that bind the target molecule can be recovered; individual phage can be cloned, and the peptide expressed by a cloned phage can be determined.

[0073] Similarly, Smith and Scott (*Meth. Enzymol.* 217: 228-257 (1993) and *Science* 249: 386-390 (1990)) describe methods of producing phage peptide display libraries, including vectors and methods of diversifying the population of peptides that are expressed (see, also, Huse, WO 91/07141 and WO 91/07149). Phage display technology can be particularly powerful when used, for example, with a codon based mutagenesis method, which can be used to produce random peptides or desirably biased peptides (see,

[0076] Other carriers for stimulating or enhancing an immune response include cytokines such as IL-1, IL-1 α and β peptides, IL-2, γ -INF, IL-10, GM-CSF, and chemokines, such as macrophage inflammatory protein (MIP)1 α and β and RANTES (i.e., regulation upon activation normal T cell expressed and secreted). Immunogenic agents can also be linked to peptides that enhance transport across tissues, as described in WO 97/17613 and WO 97/17614.

[0077] Immunogenic agents can be linked to carriers by chemical cross-linking. Techniques for linking an agent to a carrier include but are not limited to the formation of disulfide linkages using N-succinimidyl-3-(2-pyridyl-thio) propionate (SPDP) and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) (if the peptide lacks

a sulfhydryl group, this can be provided by addition of a cysteine residue). These reagents create a disulfide linkage between themselves and a peptide cysteine that resides on one protein and an amide linkage through the ϵ -amino on a lysine, or other free amino group in other amino acids. A variety of such disulfide/amide-forming agents are described in *Immun. Rev.* 62: 185 (1982). Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thio-ether-forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic acid, 2-iodoacetic acid, and 4-(N-maleimido-methyl)cyclohexane-1-carboxylic acid. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxyl-2-nitro-4-sulfonic acid sodium salt.

[0078] Peptide agents can also be expressed as fusion proteins with carriers (i.e., heterologous peptides). The peptide agent can be linked at its amino terminus, its carboxyl terminus, or both to a carrier. Optionally, multiple repeats of the immunogenic peptide can be present in the fusion protein. Optionally, an immunogenic peptide can be linked to multiple copies of a heterologous peptide, for example, at both the N- and C-termini of the peptide. Some carrier peptides serve to induce a helper T-cell response against the carrier peptide. The induced helper T-cells in turn induce a B-cell response against the immunogenic peptide linked to the carrier peptide.

[0079] Whether the agent is an antibody, polypeptide, peptide, small molecule or other pharmaceutically useful compound according to the present invention that is to be given to an individual, administration is via a chronic dosage regime. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g., decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in *Remington's Pharmaceutical Sciences*, 18th edition, Osol, A. (ed.), 1990.

Pharmaceutical Compositions

[0080] The present invention also provides pharmaceutical compositions for the reduction of chronic pathological inflammation in a subject susceptible to such and/or suffering from a disorder associated with pathological inflammation.

[0081] Pharmaceutical formulations of the invention preferably contain an agent in a concentration from about 0.1 to about 10% of the formulation. They may also be used in appropriate association with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way meant to be limiting.

[0082] For oral preparations, the agents can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or

magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

[0083] When the agent is an antibody, the formulation is preferably administered in a parenteral dosage form. The preferred form depends on the intended mode of administration and therapeutic application. The compositions can also include, depending on the formulation desired, pharmaceutically acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, non-immunogenic stabilizers and the like. Also included may be carrier molecules such as proteoglycans. Specific examples of such carrier molecules include, but are not limited to, glycosaminoglycans such as heparin sulfate, hyaluronic acid, keratan sulfate, chondroitin 4-sulfate, chondroitin 6-sulfate, heparan sulfate and dermatin sulfate, perlecan, and pentopolysulfate.

[0084] Antibodies of the invention can be administered as injectable dosages of a solution or as a suspension of the substance in a physiologically acceptable diluent with a pharmaceutical carrier that can be a sterile liquid such as water and oils with or without the addition of a surfactant. Other pharmaceutically preparations are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, and mineral oil. In general, glycols such as propylene glycol or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions. The agents of this invention can be administered in a sustained release form, for example a depot injection, implant preparation, or osmotic pump, which can be formulated in such a manner as to permit a sustained release of the active ingredient.

[0085] In addition, agents of the invention that are antibodies may be provided by administering a polynucleotide encoding a whole or partial antibody (e.g., a single chain Fv) to a subject. The polynucleotide is administered to a subject in an appropriate vehicle to allow the expression of the antibody in the subject in a therapeutically effective amount.

[0086] The agents of the invention can be formulated into preparations for injections by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol. The formulations may also contain conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[0087] The agents can be utilized in aerosol formulation to be administered via inhalation or pulmonary delivery. The agents of the present invention can be formulated under pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

[0088] Furthermore, the agents can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. The agents of the present invention can be administered rectally via a suppository. The

suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

[0089] Administration of an agent of the invention may be accomplished by any convenient means, including parenteral injection, and may be systemic or localized in delivery. The agents of this invention can be incorporated into a variety of formulations for therapeutic administration. More particularly, the agents of the present invention can be formulated into pharmaceutical compositions by combination with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. As such, administration of the agents can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intratracheal, intranasal, gastric, intramuscular, intracranial, subdermal etc., administration. The active agent may be systemic after administration or may be localized by the use of regional administration, intramural administration, or use of an implant that acts to retain the active dose at the site of implantation.

[0090] Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more agents of the present invention. Similarly, unit dosage forms for injection or intravenous administration may comprise the agent of the present invention in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

[0091] Implants for sustained release formulations are well known in the art. Implants are formulated as microspheres, slabs, etc. with biodegradable or non-biodegradable polymers. For example, polymers of lactic acid and/or glycolic acid form an erodible polymer that is well tolerated by the host. The implant is placed in proximity to the site of protein deposits (e.g., the site of formation of amyloid deposits associated with neurodegenerative disorders), so that the local concentration of active agent is increased at that site relative to the rest of the body.

[0092] A typical dosage unit for administration of a subject includes, but is not limited to: a solution suitable for intravenous administration; a tablet taken from two to six times daily; or a one time-release capsule or tablet taken once a day and containing a proportionally higher content of active ingredient, etc. The time-release effect may be obtained by capsule materials that dissolve at different pH values, by capsules that release slowly by osmotic pressure or by any other known means of controlled release.

[0093] Certain agents of the invention, including antibodies and peptides, are sometimes administered in combination with an adjuvant. A variety of adjuvants can be used in combination with an anti alpha-4 integrin agent to elicit an immune response. Preferred adjuvants augment the intrinsic response to an agent without causing conformational changes in the agent that affect the qualitative form of the response. Preferred adjuvants include aluminum hydroxide and aluminum phosphate, 3 De-O-acylated monophosphoryl

lipid A (MPL™) (see GB 2220211 (RIBI ImmunoChem Research Inc., Hamilton, Mont., now part of Corixa). Stimulon™ QS-21 is a triterpene glycoside or saponin isolated from the bark of the Quillaja Saponaria Molina tree found in South America (see Kensil et al., in *Vaccine Design: The Subunit and Adjuvant Approach* (eds. Powell & Newman, Plenum Press, NY, 1995); U.S. Pat. No. 5,057,540, (Aquila BioPharmaceuticals, Framingham, Mass.). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (see Stoute et al., 1997 N. Engl. J. Med. 336: 86-91). Another adjuvant is CpG (WO 98/40100). Alternatively, an agent can be coupled to an adjuvant. However, such coupling should not substantially change the conformation of the desired alpha-4 epitope so as to affect the nature of the host immune response. Adjuvants can be administered as a component of a therapeutic composition with an active agent or can be administered separately, before, concurrently with, or after administration of the therapeutic agent.

[0094] A preferred class of adjuvants for administration is aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, and aluminum sulfate. Such adjuvants can be used with or without other specific immunostimulating agents such as MPL or 3-DMP, QS-21, polymeric or monomeric amino acids such as polyglutamic acid or polylysine. Another class of adjuvants is oil-in-water emulsion formulations. Such adjuvants can be used with or without other specific immunostimulating agents such as muramyl peptides (e.g., N-acetylmuramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), N-acetylglucosaminyl-N-acetylmuramyl-L-Al-D-isoglu-L-Ala-dipalmitoxy propylamide (DTP-DPP) theramide™, or other bacterial cell wall components. Oil-in-water emulsions include (a) MF59 (WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton Mass.), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, Mont.) containing 2% squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphoryl lipid A, trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL+CWS (Detox™). Another class of preferred adjuvants is saponin adjuvants, such as Stimulon™ (QS-21; Aquila, Framingham, Mass.) or particles generated therefrom, such as ISCOMs (immunostimulating complexes) and ISCOMATRIX. Other adjuvants include Complete and Incomplete Freund's Adjuvant (IFA), cytokines, such as interleukins (IL-1, IL-2, and IL-12), macrophage colony stimulating factor (M-CSF), and tumor necrosis factor (TNF). Such adjuvants are generally available from commercial sources.

[0095] An adjuvant can be administered with an agent as a single composition, or can be administered before, concurrent with or after administration of the agent. The agent and an adjuvant can be packaged and supplied in the same vial or can be packaged in separate vials and mixed before

use. The agent and adjuvant are typically packaged with a label indicating the intended therapeutic application. If the agent and adjuvant are packaged separately, the packaging typically includes instructions for mixing before use. The choice of an adjuvant and/or carrier depends on such factors as the stability of the formulation containing the adjuvant, the route of administration, the dosing schedule, and the efficacy of the adjuvant for the species being vaccinated. In humans, a preferred pharmaceutically acceptable adjuvant is one that has been approved for human administration by pertinent regulatory bodies. Examples of such preferred adjuvants for humans include alum, MPL and QS-21. Optionally, two or more different adjuvants can be used simultaneously. Preferred combinations include alum with MPL, alum with QS-21, MPL with QS-21, and alum, QS-21 and MPL together. Also, Incomplete Freund's adjuvant can be used (Chang et al., 1998 *Advanced Drug Delivery Reviews* 32: 173-186), optionally in combination with any of alum, QS-21, and MPL and all combinations thereof.

Chronic Administration Dosage Regimes

[0096] The chronic treatment regimes of the present invention provides anti-alpha-4 integrin agent at a level that will maintain sufficient receptor saturation to suppress pathological inflammation in a patient in need of such. The methods of the invention entail administration once per every two weeks or once a month to once every two months, with repeated dosings taking place over a period of at least six months, and more preferably for a year or longer. The methods of the invention involve obtaining and maintaining a receptor saturation level in a human patient of a dimer comprising alpha-4 integrin (e.g., VLA-4) in a range of from about 65% to 100%, more preferably between about 75% to about 100%, and even more preferably between about 80% to about 100%. These receptor saturation levels are maintained at these levels chronically (e.g., over a period of 6 months or so) to allow for continued suppression of pathological inflammation.

[0097] In a specific embodiment, the anti-alpha-4 agent is an antibody, preferably a humanized or human antibody (e.g., natalizumab), and the dosing is on a monthly basis. Levels of receptor saturation can be monitored to determine the efficacy of the dosing regime, and physiological markers measured to confirm the success of the dosage regime. As a confirmation, serum levels of the antibody can be monitored to identify clearance of the antibody and to determine the potential effect of half-life on the efficacy of the treatment.

[0098] The amount of agent administered in a dosage unit may depend on whether adjuvant is also administered, with higher dosages generally being required in the presence of adjuvant. For immunization with an agent of the invention, the dosage ranges from about 0.0001 to about 100 mg/kg, and more usually about 0.01 to 5 mg/kg, of the host body weight. For example, dosages can be about 1 mg/kg body weight or about 10 mg/kg body weight. Dosage and frequency may vary depending on the half-life of the agent in the patient. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. For antibody administration, each dosing injection is generally between about 2.0 to about 8.0 mg/kg. In accordance with the teachings provided herein, effective dosages can be monitored by obtaining a fluid sample from the patient, generally a blood serum or cerebrospinal fluid

sample, and determining the integrin receptor saturation using methods well known in the art. Ideally, a sample is taken prior to initial dosing; subsequent samples are taken and measured prior to and/or after each immunization. A particularly preferred amount is a 3 mg per kg of patient per month of natalizumab or an immunologically active fragment equivalent thereof.

[0099] When adjuvant is being administered, the dosage level is increased in accordance with the particular adjuvant and the level of immunogenicity of the anti-alpha-4 agent. Doses for individual agents, selected in accordance with the present invention, are determined according to standard dosing methods, taken in conjunction with the teachings provided herein.

[0100] As an alternative to chronic administration comprised of repeated individual dosings, an anti-alpha-4 agent can be administered as a sustained release formulation, provided the dosage is such that the levels of receptor saturation remain sufficient to suppress inflammation. For example, controlled release systems can be used to chronically administer an anti-alpha-4 agent within the scope of this invention. Discussions of appropriate controlled release dosage forms may be found in Leszczek Krowczynski, *Extended-Release Dosage Forms*, 1987 (CRC Press, Inc.).

[0101] The various controlled release technologies cover a very broad spectrum of drug dosage forms. Controlled release technologies include, but are not limited to physical systems and chemical systems. Physical systems include, but are not limited to, reservoir systems with rate-controlling membranes, such as microencapsulation, macroencapsulation, and membrane systems; reservoir systems without rate-controlling membranes, such as hollow fibers, ultra microporous cellulose triacetate, and porous polymeric substrates and foams; monolithic systems, including those systems physically dissolved in non-porous, polymeric, or elastomeric matrices (e.g., non-erodible, erodible, environmental agent ingress, and degradable), and materials physically dispersed in non-porous, polymeric, or elastomeric matrices (e.g., non-erodible, erodible, environmental agent ingress, and degradable); laminated structures, including reservoir layers chemically similar or dissimilar to outer control layers; and other physical methods, such as osmotic pumps, or adsorption onto ion-exchange resins.

[0102] Chemical systems include, but are not limited to, chemical erosion of polymer matrices (e.g., heterogeneous, or homogeneous erosion), or biological erosion of a polymer matrix (e.g., heterogeneous, or homogeneous). Additional discussion of categories of systems for controlled release may be found in Agis F. Kydonieus, *Controlled Release Technologies: Methods, Theory and Applications*, 1980 (CRC Press, Inc.).

[0103] The methods of the invention can be used to treat a patient that is affected with a disorder involving or arising from pathological inflammation, or to prophylactically treat a patient at risk for a particular disorder. The dosage regimes that are necessary for prophylactic versus therapeutic treatment may vary, and will need to be designed for the specific use and disorder treated.

[0104] In some methods, two or more agents (e.g., monoclonal antibodies with different binding specificities) are administered simultaneously, in which case the dosage of

each agent administered falls within the ranges indicated. Intervals can also be irregular as indicated by measuring receptor saturation levels or by following other indicia of the disease process.

[0105] Those of skill will readily appreciate that dose levels can vary as a function of the specific agent, the severity of the symptoms and the susceptibility of the subject to side effects. Some of the specific agents are more potent than others. Preferred dosages for a given agent are readily determinable by those of skill in the art by a variety of means. A preferred means is to measure the physiological potency of a given agent.

Therapeutic Indications

[0106] The controlled release formulations of the present invention can be used to obtain a wide range of desirable effects. Particularly the formulations of the invention are useful in treating essentially any disease state or symptom that is treatable by long term administration of anti-inflammatories that target pathological inflammation.

[0107] The invention also provides methods of treatment that exploit the ability of anti-alpha-4 integrin agents to block alpha-4-dependent interactions. The alpha-4-dependent interaction with the VCAM-1 ligand on endothelial cells is an early event in many inflammatory responses, including those of the central nervous system. Undesired diseases and conditions resulting from inflammation and having acute and/or chronic clinical exacerbations include multiple sclerosis (Yednock et al., 1992 *Nature* 356: 63; Baron et al., 1993 *J. Exp. Med.* 177: 57), meningitis, encephalitis, stroke, other cerebral traumas, inflammatory bowel disease (IBD) including ulcerative colitis and Crohn's disease (CD) (Hamann et al., 1994 *J. Immunol.* 152: 3238), (Podolsky et al., 1993 *J. Clin. Invest.* 92: 372), rheumatoid arthritis (van Dinther-Jansson et al., 1991 *J. Immunol.* 147: 4207; van Dinther-Janssen et al., 1993 *Annals Rheumatic Diseases* 52: 672); Elices et al., 1994 *J. Clin. Invest.* 93: 405); Postigo et al., 1992 *J. Clin. Invest.* 89: 1445), asthma (Mulligan et al., 1993 *J. Immunol.* 150: 2407) and acute juvenile onset diabetes (Type 1) (Yang et al., 1993 *PNAS* 90: 10494); Burkly et al., 1994 *Diabetes* 43: 529); Baron et al., 1994 *J. Clin. Invest.* 93: 1700), AIDS dementia (Sasseville et al., 1994 *Am. J. Path.* 144: 27); atherosclerosis (Cybulsky et al., 1991 *Science* 251: 788-91, Li et al., 1993 *Arterioscler. Thromb.* 13: 197), nephritis (Rabb et al., 1995 *Springer Semin. Immunopathol.* 16: 417-25), retinitis, atopic dermatitis, psoriasis, myocardial ischemia, chronic prostatitis, complications from sickle cell anemia, lupus erythematosus, and acute leukocyte-mediated lung injury such as occurs in adult respiratory distress syndrome.

[0108] Inflammatory bowel disease is a collective term for two similar diseases referred to as Crohn's disease (CD) and ulcerative colitis. CD is an idiopathic, chronic ulceroinflammatory disease characterized by sharply delimited and typically transmural involvement of all layers of the bowel wall by a granulomatous inflammatory reaction. Any segment of the gastrointestinal tract, from the mouth to the anus, may be involved, although the disease most commonly affects the terminal ileum and/or colon. Ulcerative colitis is an inflammatory response limited largely to the colonic mucosa and submucosa. Lymphocytes and macrophages are numerous in lesions of inflammatory bowel disease and may contribute to inflammatory injury.

[0109] Asthma is a disease characterized by increased responsiveness of the tracheobronchial tree to various stimuli potentiating paroxysmal constriction of the bronchial airways. The stimuli cause release of various mediators of inflammation from IgE-coated mast cells including histamine, eosinophilic and neutrophilic chemotactic factors, leukotrienes, prostaglandin and platelet activating factor. Release of these factors recruits basophils, eosinophils and neutrophils, which cause inflammatory injury.

[0110] Atherosclerosis is a disease of arteries (e.g., coronary, carotid, aorta and iliac). The basic lesion, the atheroma, consists of a raised focal plaque within the intima, having a core of lipid and a covering fibrous cap. Atheromas compromise arterial blood flow and weaken affected arteries. Myocardial and cerebral infarcts are a major consequence of this disease. Macrophages and leukocytes are recruited to atheromas and contribute to inflammatory injury.

[0111] Rheumatoid arthritis is a chronic, relapsing inflammatory disease that primarily causes impairment and destruction of joints. Rheumatoid arthritis usually first affects the small joints of the hands and feet but then may involve the wrists, elbows, ankles and knees. The arthritis results from interaction of synovial cells with leukocytes that infiltrate from the circulation into the synovial lining of joints. See e.g., Paul, *Immunology* (3d ed., Raven Press, 1993).

[0112] Another indication for chronic dosage of anti alpha-4 agents is in treatment of organ or graft rejection. Over recent years there has been a considerable improvement in the efficiency of surgical techniques for transplanting tissues and organs such as skin, kidney, liver, heart, lung, pancreas and bone marrow. Perhaps the principal outstanding problem is the lack of satisfactory agents for inducing immunotolerance in the recipient to the transplanted allograft or organ. When allogeneic cells or organs are transplanted into a host (i.e., the donor and donee are different individuals from the same species), the host immune system is likely to mount an immune response to foreign antigens in the transplant (host-versus-graft disease) leading to destruction of the transplanted tissue. CD8⁺ cells, CD4⁺ cells and monocytes are all involved in the rejection of transplant tissues. Antibodies directed to alpha-4 integrin are useful, inter alia, to block alloantigen-induced immune responses in the donee thereby preventing such cells from participating in the destruction of the transplanted tissue or organ. See, e.g., Paul et al., 1996 *Transplant International* 9: 420-425; Georczynski et al., 1996 *Immunol.* 87: 573-580); Georczynski et al., 1995 *Transplant. Immunol.* 3: 55-61); Yang et al., 1995 *Transplantation* 60: 71-76); Anderson et al., 1994 *APMIS* 102: 23-27.

[0113] A related use for anti alpha-4 agents is in modulating the immune response involved in "graft versus host" disease (GVHD). See e.g., Schlegel et al., *J. Immunol.* 155, 3856-3865 (1995). GVHD is a potentially fatal disease that occurs when immunologically competent cells are transferred to an allogeneic recipient. In this situation, the donor's immunocompetent cells may attack tissues in the recipient. Tissues of the skin, gut epithelia and liver are frequent targets and may be destroyed during the course of GVHD. The disease presents an especially severe problem when immune tissue is being transplanted, such as in bone marrow transplantation; but less severe GVHD has also been

reported in other cases as well, including heart and liver transplants. The therapeutic agents of the present invention are used, inter alia, to block activation of the donor T-cells thereby interfering with their ability to lyse target cells in the host.

[0114] A further use of anti alpha-4 agents of the invention is inhibiting tumor metastasis. Several tumor cells have been reported to express alpha-4 integrin and antibodies to alpha-4 integrin have been reported to block adhesion of such cells to endothelial cells (Steinback et al., 1995 *Urol. Res.* 23: 175-83); Orosz et al., 1995 *Int. J. Cancer* 60: 867-71); Freedman et al., 1994 *Leuk. Lymphoma* 13: 47-52); Okahara et al., 1994 *Cancer Res.* 54: 3233-6).

[0115] A further use of the anti-alpha-4 agents is in treating multiple sclerosis. Multiple sclerosis (MS) is a progressive neurological autoimmune disease that affects an estimated 250,000 to 350,000 people in the United States. Multiple sclerosis is thought to be the result of a specific autoimmune reaction in which certain leukocytes attack and initiate the destruction of myelin, the insulating sheath covering nerve fibers. In an animal model for multiple sclerosis, murine monoclonal antibodies directed against alpha-4 beta-1 integrin have been shown to block the adhesion of leukocytes to the endothelium, and thus prevent inflammation of the central nervous system and subsequent paralysis in the animals.

[0116] The onset of MS may be dramatic or so mild as to not cause a patient to seek medical attention. The most common symptoms include weakness (in one or more limbs, visual blurring due to optic neuritis, sensory disturbances, diplopia and ataxia. The course of disease may be stratified into three general categories: (1) relapsing MS, (2) chronic progressive MS, and (3) inactive MS. Relapsing MS is characterized by recurrent attacks of neurologic dysfunction. MS attacks generally evolve over days to weeks and may be followed by complete, partial or no recovery. Recovery from attacks generally occurs within weeks to several months from the peak of symptoms, although rarely some recovery may continue for 2 or more years.

[0117] Chronic progressive MS results in gradually progressive worsening without periods of stabilization or remission. This form develops in patients with a prior history of relapsing MS, although in 20% of patients, no relapses can be recalled. Acute relapses also may occur during the progressive course.

[0118] A third form is inactive MS. Inactive MS is characterized by fixed neurologic deficits of variable magnitude. Most patients with inactive MS have an earlier history of relapsing MS.

[0119] The course of MS is also dependent on the age of the patient. For example, favourable prognostic factors include early onset (excluding childhood), a relapsing course and little residual disability 5 years after onset. By contrast, poor prognosis is associated with a late age of onset (i.e., age 40 or older) and a progressive course. These variables are interdependent, since chronic progressive MS tends to begin at a later age than relapsing MS. Disability from chronic progressive MS is usually due to progressive paraplegia or quadriplegia in individual patients. In one aspect of the invention, patients will preferably be treated when the patient is in remission rather than in a relapsing stage of the disease.

[0120] Short-term use of either adrenocorticotrophic hormone (ACTH) or oral corticosteroids (e.g., oral prednisone or intravenous methylprednisolone) is the only specific therapeutic measure for treating patients with acute exacerbation of MS.

[0121] Newer therapies for MS include treating the patient with interferon beta-1b, interferon beta-1a, and copaxone® (formerly known as copolymer 1). These three drugs have been shown to significantly reduce the relapse rate of the disease. These drugs are typically self-administered intramuscularly or subcutaneously.

[0122] None of the currently available treatments inhibit demyelination or MS. One aspect of the invention contemplates treating MS with agents disclosed herein either alone or in combination with other standard treatment modalities. Standard treatment modalities include but are not limited to the following. Additional treatment modalities not discussed herein for use in treating MS in combination with the methods and compositions disclosed herein depending on the state of disease in the patient would be evident to the skilled practitioner. Such additional treatment modalities for MS other pathological inflammation would include other immunomodulators or immunosuppressants.

[0123] The agents and pharmaceutical compositions discussed supra can be chronically administered for prophylactic and/or therapeutic treatments of the previously listed inflammatory disorders, including multiple sclerosis, inflammatory bowel disease, asthma, atherosclerosis, rheumatoid arthritis, organ or graft rejection and graft versus host disease. In therapeutic applications, compositions are administered to a patient suspected of, or already suffering from such a disease in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as a therapeutically- or pharmaceutically-effective dose.

[0124] In prophylactic applications, pharmaceutical compositions are chronically administered to a patient susceptible to, or otherwise at risk of, a particular disease in an amount sufficient to eliminate or reduce the risk or delay the onset of the disease. Such an amount is defined to be a prophylactically effective dose. In patients with multiple sclerosis in remission, risk may be assessed by NMR imaging or, in some cases, by presymptomatic indications observed by the patient.

[0125] Effective dosage regimes of the compositions of the present invention, for the treatment of the above described conditions will vary depending upon many different factors, including means of administration, target site, physiological state of the patient, and other medicaments administered. Thus, treatment dosages will need to be titrated to optimize safety and efficacy. In general, each administration of the dosage regime will range from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg of the host body weight. One preferred dosage regimen is 300 mg administered once per month for a period of at least 6 months, more preferably 12 months and perhaps over the course of several years. Another dosage regimen that is preferred is a 3 mg per kilogram of patient weight per month. Such a regimen may be preferable for pediatric or adolescent patients in need of therapy.

Combination Therapies

[0126] The anti-alpha-4 agents of the invention can be used with effective amounts of other therapeutic agents against acute and chronic inflammation. Such agents include other antagonists of adhesion molecules (e.g., other integrins, selectins, and immunoglobulin (Ig) super family members (see Springer, 1990 *Nature* 346: 425-433; Osborn, 1990 *Cell* 62: 3; Hynes, 1992 *Cell* 9: 11). Integrins are heterodimeric transmembrane glycoproteins consisting of an α chain (120-180 kDa) and a β chain (90-110 kDa), generally having short cytoplasmic domains. For example, three important integrins (i.e., LFA-1, Mac-1 and P150,95) have different alpha subunits, designated CD11a, CD11b and CD11c, and a common beta subunit designated CD18. LFA-1 ($\alpha_1\beta_2$) is expressed on lymphocytes, granulocytes and monocytes, and binds predominantly to an Ig-family member counter-receptor termed ICAM-1 and related ligands. ICAM-1 is expressed on many cells, including leukocytes and endothelial cells, and is up-regulated on vascular endothelium by cytokines such as TNF and IL-1. Mac-1 ($\alpha_M\beta_2$) is distributed on neutrophils and monocytes, and also binds to ICAM-1. The third β_2 integrin, P150,95 ($\alpha_X\beta_2$), is also found on neutrophils and monocytes. The selectins consist of L-selectin, E-selectin and P-selectin.

[0127] Other anti-inflammatory agents that can be used in combination with the anti-alpha-4 agents include antibodies and other antagonists of cytokines, such as interleukins IL-1 through IL-13, tumor necrosis factors α and β , interferons α , β and γ , tumor growth factor beta (TGF- β), colony stimulating factor (CSF) and granulocyte monocyte colony stimulating factor (GM-CSF). Other anti-inflammatory agents include antibodies and other antagonists of chemokines such as MCP-1, MIP-1 α , MIP-1 β , RANTES, exotaxin and IL-8. Other anti-inflammatory agents include NSAIDS, steroids and other small molecule inhibitors of inflammation. Formulations, routes of administration and effective concentrations of agents for combined therapies are as described above for the humanized antibodies against alpha-4 integrin.

[0128] Additional agents for use in combination with agents which mediate alpha-4 integrin or dimers comprising alpha-4 integrin and treat inflammatory bowel disease (IBD), Crohn's Disease (CD) and ulcerative colitis (UC), include but are not limited to 5-aminosalicylates, glucocorticoids, thioguanine derivatives, methotrexate (MTX), cyclosporine, antibiotics, and infliximab.

[0129] 5-Aminosalicylates include sulfasalazine (also known as Azulfidine) which is a conjugate of mesalamine linked to sulfapyridine by a diazo bond and is usually administered in an amount of 500 mg/day to about 6 g/day. 5-Aminosalicylates can also be co-administered with a glucocorticoid. Preferably, a 5-aminosalicylate is used in combination therapy with one of the other agents discussed herein to treat ulcerative colitis, however it can also be used to treat Crohn's disease. Non-sulfonamide containing formulations of mesalamine include but are not limited to ASACOL®, CLAVERSA, SALOFALK, PENTASA®, DIPENTUM®, COLAZIDE and ROWASA®.

[0130] Glucocorticoids have been a mainstay of treatment for acute severe exacerbations of IBD since 1955, when they first were shown to be efficacious in UC. Oral prednisone can be administered in conjunction with any of the agents disclosed herein. Typically, 20 to 40 mg of oral prednisone

is administered once a day. Glucocorticoids can also be administered intravenously and via enemas in combination with or concurrently with or within a short time before/after an anti-alpha-4 integrin agent is administered. For example, hydrocortisone is available as a retention enema (100 mg/60 mL) and the usual dose is one 60-mL enema per night for 2 to 3 weeks. This can be altered when used in combination with the therapies and agents discussed herein as would be understood by the artisan of ordinary skill. Other steroids that can be used include, but are not limited to, prednisolone methanesulfobenzoate, tixocortol pivalate, fluticasone propionate, beclomethasone dipropionate, and budesonide.

[0131] Thioguanine derivatives are also useful in the treatment of IBD, CD and UC. These include but are not limited to 6-mercaptopurine (6-MP) and azathioprine (IMURAN). The two drugs can be used interchangeably in combination with any of the alpha-4 integrin modulating agents discussed herein.

[0132] Methotrexate (MTX) is also contemplated for use in combination with the alpha-4 integrin regulatory agents discussed herein. Preferably, MTX is administered via intramuscular injection (i.m.) to the subject in combination with an anti-alpha-4 integrin agent. MTX is effective in steroid-dependent CD, but not as useful in UC. MTX can be administered in amounts of about 15 to about 25 mg per week per subject or as necessary as determined by the artisan of ordinary skill.

[0133] Cyclosporines (e.g., SANDIMMUNE®, NEORAL®) can also be used in combination with the alpha-4 integrin modulating agents discussed herein to treat pathological inflammation of the bowel. This can be used to treat acute, severe UC, which does not respond to glucocorticoids.

[0134] Infliximab (i.e., REMICADE®) can also be used to treat CD in combination with the alpha-4 integrin modulating agents indicated herein. Infliximab is an immunoglobulin that binds to TNF and thereby neutralizes its activity. Other anti-TNF antibodies, such as CDP571, can also be used in combination with the alpha-4 integrin modulating agents disclosed herein.

[0135] Antibiotics are also contemplated for use in combination with the alpha-4 integrin modulating agents indicated herein to modulate UC, IBD and CD. For example, patients can be treated with metronidazole or ciprofloxacin (or pharmacological equivalents thereof) in combination with an alpha-4 integrin mediating agent or in the form of an admixture.

[0136] Also contemplated is the use of supportive therapies for IBD, CD and UC in conjunction with agents that mediate alpha-4 integrin or dimers comprising alpha-4 integrin and treat inflammatory bowel disease, Crohn's Disease and ulcerative colitis. Supportive therapies include, but are not limited to, analgesics, anticholinergic and antidiarrheal agents. Combining such supportive therapies can be useful in the beginning of a treatment regimen in reducing a patient's symptoms and improving their quality of life. Supportive therapies include administering oral iron, folate, and vitamin B₁₂. Antidiarrheal agents include, but are not limited to diphenoxylate, codeine, loperamide, and anticholinergics (or pharmacological equivalents thereof), which can be administered to patients with mild disease to reduce

the frequency of bowel movements and relieve rectal urgency. Cholestyramine can be used in patients to prevent bile salt-induced colonic secretion in patients who have already undergone limited ileocolic resections prior to treatment with the chronic regimens described herein. Anticholinergic agents include, but are not limited to, clidinium bromide, dicyclomine hydrochloride, tincture of belladonna and the like, and are useful to reduce abdominal cramps, pain and rectal urgency.

[0137] For treatment of MS, the anti-alpha-4 integrin agents (e.g., anti-alpha-4 integrin antibodies, small compound alpha-4 integrin antagonists and the like) can be combined with other compounds or compositions used to treat, ameliorate or palliate symptoms associated with MS.

[0138] Other agents utilized to treat, ameliorate or palliate symptoms associated with MS, include but are not limited to: muscle relaxants (e.g., Diazepam, cyclobenzaprine, Clonazepam, clonidine, primidone, and the like), anticholinergics (e.g., propantheline, dicyclomine, and the like), central nervous system stimulants (e.g., Pemoline), non-steroidal anti-inflammatory agents (NS such as ibuprofen, naproxen and ketoprofen), interferons, immune globulin, glatiramer (Copaxone®), mitoxantrone (Novantrone®), misoprostol, tumor necrosis factor-alpha inhibitors (e.g., Pirfenidone, infliximab and the like) and corticosteroids (e.g., glucocorticoids and mineralocorticoids).

[0139] Common agents for treating multiple sclerosis include interferon beta-1b (Betaseron®), interferon beta-1a (Avonex®), high-dose interferon beta-1a (Rebif®), Glatiramer (Copaxone®), immune globulin, mitoxantrone (Novantrone®), corticosteroids (e.g., prednisone, methylprednisolone, dexamethasone and the like). Other corticosteroids may also be used and include but are not limited to cortisol, cortisone, fludrocortisone, prednisolone, 6 α -methylprednisolone, triamcinolone, and betamethasone.

[0140] Dosage forms of the agents to be used in combination with the compounds and compositions disclosed herein would vary depending on the subject and drug combination being utilized. For example, interferons are typically administered as follows: Interferon beta-1a (Avonex®) is administered 30 μ g once a week; interferon beta-1a is administered at about 22 μ g or 44 μ g three times a week; and interferon beta-1b (Betaseron®) is administered at 250 μ g on alternate days (Durelli et al., *Lancet* 359: 1453-60, 2002). Typically the interferons are administered for relapsing or remitting multiple sclerosis. Thus in combination with the anti-alpha-4 integrin agents disclosed herein, preferred ranges of interferons may include about 0.1 μ g to about 250 μ g and more preferably about 0.5 μ g to about 50 μ g, depending on the manner in which the agent is administered in conjunction with the other anti-alpha-4 integrin compounds and compositions disclosed herein.

[0141] NS or NSAIDs contemplated for use with this invention include but are not limited to non-selective COX inhibitors and selective COX-2 inhibitors. Non-selective COX inhibitors include but are not limited to salicylic acid derivatives (e.g., aspirin, sodium salicylates, choline magnesium trisalicylate, salsalate, diflunisal, sulfasalazine, and olsalazine), para-aminophenol derivatives (e.g., acetaminophen), indole and indene acetic acids (e.g., tolmetin, diclofenac, and ketorolac), heteroaryl acetic acids (e.g., abuprofen, naproxen, flurbiprofen, ketoprofen, fenpropfen,

and oxaprozin), anthranilic acids or fenamates (e.g., mefenamic acid and meclofenamic acid), enolic acids (e.g., oxicams such as piroxicam and meloxicam), and alkanones (e.g., nabumetone). Selective COX-2 inhibitors include diaryl-substituted furanones (e.g., rofecoxib), diaryl-substituted pyrazoles (e.g., celecoxib), indole acetic acids (e.g., etodolac), and sulfonanilides (e.g., nimesulide). NS are often-times administered in combination with interferon to lessen the flu-like symptoms experienced by patients receiving, for example, Avonex®. Common NS agents include naproxen, ibuprofen and ketoprofen. Paracetamol is also frequently administered to patients. See, Reess et al., 2002 *Mult. Scler.* 8: 15-8.

[0142] Glatiramer acetate (GA, Copaxone®) is a synthetic molecule that inhibits activation of myelin basic protein-reactive T cells and induces a T-cell repertoire characterized by anti-inflammatory effects. Moreover, Glatiramer can access the central nervous system (CNS), whereas interferon-beta cannot (Dhib-Jalbut, 2002 *Neurology* 58: S3-9; Weinstock-Guttman et al., 2000 *Drugs* 59: 401-10).

[0143] Mitoxantrone is an anthracenedione synthetic agent, which has been shown to be effective for treating secondary progressive multiple sclerosis (SP-MS). However, use of this drug is again limited by its cumulative cardiotoxicity (Weinstock-Guttman et al., 2000).

[0144] Tumor necrosis factor-alpha (TNF- α) may be a key cytokine in demyelination (Walker et al., 2001 *Mult. Scler.* 7: 305-12). Thus use of agents that antagonize TNF- α function or inhibit its synthesis may be useful in combination with the agents and compounds disclosed herein. This can include anti-TNF- α antibodies (e.g., infliximab) as well as agents such as pirfenidone. Pirfenidone is a non-peptide drug, which has been shown to decrease synthesis of TNF- α and to block receptors for TNF- α . Id.

[0145] The long mainstay in most demyelinating conditions and diseases has been the use of ACTH, glucocorticoids and corticoid steroids. These agents are used for their anti-edema and anti-inflammatory effects. ACTH is commonly administered to a subject at 80 U given intravenously in 500 mL of 5% dextrose and water over 6-8 hours for 3 days. It may also be administered at 40 U/ml intramuscularly at a dose of 40 U every 12 hours for 7 days, with the dose then reduced every 3 days. See, S. Hauser, "Multiple sclerosis and other demyelinating diseases," in *Harrison's Principles of Internal Medicine* 2287-95 (13th ed., Isselbacher et al., ed. 1994). Methylprednisolone is typically administered slowly in 500 ml D5W over 6 hours, preferably in the morning. Common dosages include 1000 mg daily for 3 days, 500 mg daily for 3 days and 250 mg daily for 3 days. Id. A methylprednisolone-prednisone combination is also commonly administered. Typically about 1,000 mg of intravenous methylprednisolone is administered over three days followed by oral prednisone at 1 mg/kg per day for 14 days. Thus, for use in combination with the compounds and compositions disclosed herein, the steroids may be administered in amounts ranging from about 1 to about 1,000 mg/kg over about 1 to 14 days, as needed.

[0146] A side effect in demyelinating conditions such as MS, is fatigue and decreased cognitive function. Agents such as amantadine hydrochloride and pemoline have been frequently used to treat fatigue associated with MS (Geisler et al., 1996 *Arch. Neurol.* 53: 185-8).

[0147] The benefit of such combination therapies is that it may lessen the class-specific and agent-specific side effects currently encountered with some of the drugs. Class-specific side effects of interferon-beta include fever, chills, myalgias, arthralgias and other flu-like symptoms beginning 2-6 hours after injection and typically resolving 24 hours post injection. Occasionally interferon-beta also induces transient worsening of preexisting MS symptoms. Agent specific side effects include injection-site reactions with interferon beta-1b. Management of these effects can be accomplished by tailoring the dose and time of administration, prescribing appropriate combinations of acetaminophen, non-steroidal anti-inflammatory drugs (NS or NSAIDS) and steroids. See Munschauer et al., 1997 *Clin. Ther.* 19: 883-93.

[0148] Thus, combinations of drugs that can lessen the quantity of a particular drug administered may reduce adverse side effects experienced by a patient.

[0149] When administered in combination, the small compound alpha-4-integrin antagonists may be administered in the same formulation as these other compounds or compositions, or in a separate formulation. When administered in combination, the anti-alpha-4-antibodies are generally administered in a separate formulation than the other compounds and compositions. When administered in combinations, the anti-alpha-4 agents may be administered prior to, following, or concurrently with the other compounds and compositions used to treat, ameliorate, or palliate symptoms.

EXAMPLES

[0150] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of representative examples of how to make and use embodiments of the present invention, and are not intended to limit the scope of what the inventors regard as their invention, nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1

Controlled Trial of Natalizumab in Relapsing Multiple Sclerosis

[0151] Patient Population

[0152] Twenty-six clinical centers in the United States, Canada, and the United Kingdom enrolled 213 patients from September 1999 until May 2000. The institutional review board or central and local ethics committee approved the protocol. All patients gave written informed consent. Study oversight was provided by an independent safety data monitoring committee.

[0153] Eligible subjects were required to be age 18 through 65 years, with Poser criteria defined clinically or laboratory supported definite MS, either relapsing-remitting or secondary progressive, (Poser et al., 1983 *Ann. Neurol.* 13: 227-31; Lublin et al., 1996 *Neurology* 46: 907-11), a

history of at least two relapses within the previous two years, a base-line Kurtzke Expanded Disability Status Score (EDSS) (Kurtzke, 1983 *Neurology* 33: 1444-52) between 2 and 6.5, and a minimum of three lesions on T₂-weighted brain MRI. Patients were excluded if they received immunosuppressive or immunomodulating treatments within the past 3 months, or experienced a relapse, or received systemic corticosteroids within the past 30 days.

[0154] Study Design and Randomization

[0155] Patients were randomly assigned to one of three treatment groups: 3 mg/kg natalizumab, 6 mg/kg natalizumab, or placebo according to a computer-generated block randomization schedule. Patients received six intravenous infusions at 28-day intervals and then had six months of safety follow-up. The investigator, all other study personnel, and patients were blinded to treatment assignment.

[0156] Study Procedures and Endpoints

[0157] Unenhanced proton density T₂-weighted and Gd-enhanced T₁-weighted MRI brain scans were obtained during the screening phase (month-1), immediately before each treatment (month 0-5), and one month after the last treatment (month 6). Follow-up MRI scans were obtained at months 9 and 12. Forty-six contiguous, 3-mm thick, axial slices through the brain were acquired. MRI analysis was performed by a single center blinded to patient treatment and history. Lesions were identified on hard copy images by two experienced clinicians working by consensus.

[0158] The prospective primary outcome measure was the number of new Gd-enhancing lesions over the 6-month treatment period, defined as the period following the first infusion to one month after the last infusion. Other MRI parameters evaluated included: the number of persistent Gd-enhancing lesions (enhancing lesions that had also enhanced on the previous monthly scan); the volume of Gd-enhancing lesions (measured by a semiautomated local thresholding method; Grimaud et al., 1996 *Magn. Reson. Imaging* 14: 495-505); the number of new active lesions (i.e., new Gd-enhancing lesions plus new or enlarging, non-enhancing T2 lesions); and the number of active scans (i.e., containing one or more new Gd-enhancing lesions).

[0159] Clinical endpoints included relapse frequency and changes in EDSS, and a self-reported global assessment using a visual-analog scale (VAS). All adverse events were recorded. Patients were examined at scheduled quarterly intervals, and at unscheduled visits for suspected relapses, by the treating and evaluating neurologists who were both unaware of the patient's treatment assignment. The treating neurologist performed a medical history and examination, and recorded adverse events. The evaluating neurologist assessed neurological status and assigned an EDSS score without knowledge of the patient's history or prior EDSS scores.

[0160] An objective relapse was defined as the occurrence of an acute episode of new or worsening MS symptoms lasting at least 48 hours following a stable period of at least 30 days. It was also accompanied by an increase of at least one point in the EDSS score, an increase of at least one point on two functional system scores (FSS), or an increase of at least two points on one FSS compared with base-line, as determined by the evaluating neurologist. Neurological symptoms that did not meet the above criteria for relapse,

but were assessed by the treating neurologist to constitute a relapse, were also recorded (total relapses).

[0161] On a visual analog scale (VAS), patients marked a location along a 10-cm line that reflected their assessment of their overall well-being at base-line and after 3 and 6 months treatment, with higher scores reflecting greater well-being.

[0162] Patients were followed clinically to month 12. Patients who discontinued treatment, but who did not reach the endpoint, were encouraged to return for follow-up assessments.

[0163] Statistical Analysis

[0164] Sample size estimates were based on the number of new Gd-enhancing lesions observed during the first 12 weeks following the first infusion in a previous clinical trial of natalizumab (Tubridy et al., 1999 *Neurology* 53: 466-72). Based on the results of this previous trial, and using sample size methodology appropriate for a two-sided, two-group comparison at the 5 percent level of significance, based on the Wilcoxon-Mann-Whitney statistic (Noether, 1987 *J. Amer. Stat. Assoc.* 82: 645-7), it was calculated that approximately 73 patients were needed in each group for 80 percent power.

[0165] The primary comparison of the number of Gd-enhancing lesions between 6 mg/kg natalizumab and placebo as well as Gd-enhancing volumes were evaluated with the Wilcoxon-Mann-Whitney rank-sum test. Missing values due to one or more MRI scans not being performed were imputed by replacing the missing value with the average number of lesions on available scans for that patient. MRI scans obtained from patients who received systemic corticosteroids within the previous 30 days were discarded and treated as missing values. The Cochran-Mantel-Haenszel correlation statistic, using equally spaced scores for the groups and rank scores for the primary outcome variable, was used to test for a dose-response relationship using data from all three groups.

[0166] Pearson's chi-square test was used to compare proportions of patients with relapses. Changes from baseline in EDSS and VAS were analyzed using a two-way ANOVA with study center and treatment groups as independent variables.

[0167] All analyses included all randomized patients and followed the intention-to-treat principle. All reported P values are two-tailed. There were no significant differences in demographic characteristics, MS disease history, entry EDSS, and MRI parameters among the three groups at base-line (Table 1).

TABLE 1

DEMOGRAPHIC AND BASELINE CHARACTERISTICS OF RANDOMIZED PATIENTS				
CHARACTERISTIC		PLACEBO (N = 71)	Natalizumab	
			3 MG/KG (N = 68)	6 MG/KG (N = 74)
Age, years	Mean	42.9	42.8	44.9
	Range	22-66	22-65	30-63
Gender N (%)	Male	25 (35.2)	21 (30.9)	15 (20.3)
	Female	46 (64.8)	47 (69.1)	59 (79.7)

TABLE 1-continued

DEMOGRAPHIC AND BASELINE CHARACTERISTICS OF RANDOMIZED PATIENTS				
CHARACTERISTIC		PLACEBO (N = 71)	Natalizumab	
			3 MG/KG (N = 68)	6 MG/KG (N = 74)
MS category N (%)	R-R	45 (63.4)	47 (69.1)	52 (70.3)
	S-P	26 (36.6)	21 (30.9)	22 (29.7)
EDSS	Mean	4.40	4.21	4.32
	Range	2.0-6.5	1.0-6.5	0.0-6.5
Disease duration	Mean	10.2	11.6	13.1
Years	Range	1-32	0-40	2-39
Number of relapses	Mean	3	2.9	3.1
In past 2 years	Range	2-12	2-10	2-8
Time since last relapse	Mean	6.5	7.2	6
Months	Range	2-17	2-24	2-22
Screening T ₁ -weighted MRI				
(Month 1)		28 (40)	29 (43)	29 (40)
Number (%) of scans with one or more Gd-enhancing lesion(s)				
Number of Gd-enhancing brain lesions				
	Mean	1.6	1.5	1.7
	Range	0-42	0-18	0-23
Baseline T ₁ -weighted MRI (Month 0)				
Number (%) of scans with one or more new Gd-enhancing lesion(s)		22 (31)	29 (43)	32 (43)
Number of new Gd-enhancing brain lesions				
	Mean	1.3	1.3	1.4
	Range	0-28	0-32	0-12

[0168] Primary Outcome

[0169] Patients in the placebo group exhibited an average of 9.6 new Gd-enhancing lesions during the six-month treatment period. The corresponding values in the groups receiving natalizumab were 0.7 for the 3 mg/kg group (P<0.0001) and 1.1 for the 6 mg/kg group (P<0.0001)(see Table 2). This difference constituted a 93% and an 88% reduction in new Gd-enhancing lesions in the 3 mg/kg and 6 mg/kg groups, respectively. A difference between treatment groups compared with placebo was apparent after the first infusion (FIG. 1).

TABLE 2

<u>SUMMARY OF MRI ACTIVITY DURING TREATMENT (MONTHS 1-6) AND FOLLOW - UP (MONTHS 9 AND 12)</u>				
		3	6	
	PLACEBO	MG/KG	MG/KG	P VALUE*
<u>New enhancing lesions M1-</u>				
Mean	9.6	0.7	1.1	(i) <0.0001
Median	2.0	0	0	(ii) <0.0001
SD	27.4	2.1	2.7	
<u>Persistent enhancing lesions M1-6</u>				
Mean	3.6	0.8	1.3	<0.0001
Median	1	0	0	
SD	6.5	1.9	2.6	

TABLE 2-continued

SUMMARY OF MRI ACTIVITY DURING TREATMENT (MONTHS 1-6) AND FOLLOW-UP (MONTHS 9 AND 12)				
	PLACEBO	3 MG/KG	6 MG/KG	P VALUE*
<u>New active lesions M1-6</u>				
Mean	9.7	0.8	1.1	<0.0001
Median	2.0	0	0	
SD	27.4	2.2	3	
Active scans M1-6 (%)	39%	9%	11%	(i) <0.0001 (ii) <0.0001
<u>Enhancing lesion volume M1-6 (mm³)</u>				
Mean	1169.0	156	279.0	(i) 0.005 (ii) 0.01
Median	266	0	0	
SD	2666	359.0	632.0	
<u>New enhancing lesions M9 and 12</u>				
Mean	2.5	2.6	2.1	(i) 0.90 (ii) 0.59
Median	1.0	0.5	0	
SD	4.37	4.58	4.96	
<u>Persistent enhancing lesions M9 and 12</u>				
Mean	0.2	0.1	0.1	0.029
Median	0	0	0	
SD	0.64	0.32	0.3	
<u>New active lesions M9 and 12</u>				
Mean	2.7	2.8	2.3	0.424
Median	1.0	0.5	1.0	
SD	4.49	5.69	5.11	
Active scans M9 and 12 (%)	42%	40%	35%	
<u>Enhancing lesion volume M9 and 12 (mm³)</u>				
Mean	427.0	323	233	0.260
Median	88.0	31.0	0	
SD	797.0	591.0	686	

* (i) comparison of placebo vs. 3 mg/kg natalizumab;
(ii) comparison placebo vs. 6 mg/kg natalizumab.

[0170] Secondary MRI Outcomes

[0171] There was a significant and marked reduction in the cumulative number of persistent enhancing lesions, new active lesions, total volume of enhancing lesions, and percentage of active scans from months 1-6 (Table 2; FIG. 2).

[0172] Clinical Efficacy Outcomes

[0173] During the six month treatment period, a total of 35 relapses were reported in 26 of the 71 placebo patients; 18 relapses were reported in 13 of the 68 patients receiving 3 mg/kg natalizumab, and 15 relapses were reported in 14 of the 74 patients receiving 6 mg/kg (P=0.05, placebo vs. all natalizumab-treated patients). Applying the more stringent objective relapse criteria, the effect was equally strong: 18 relapses in 15 placebo patients; 3 relapses in 3 patients receiving 3 mg/kg natalizumab; 8 relapses in 8 patients receiving 6 mg/kg natalizumab (P=0.05). More relapses in the placebo group required steroid treatment than in the treated arms (22 in placebo, 5 in the 3 mg/kg natalizumab, and 7 in the 6 mg/kg natalizumab groups, P=0.007, placebo vs. all natalizumab-treated patients).

[0174] On the VAS, patients in the placebo group reported no change, while those in the natalizumab groups reported

an improvement in well-being by month 6, at which time the difference between groups was significant (P=0.033, placebo vs. all natalizumab-treated patients). No significant changes in EDSS were observed in any group during treatment.

[0175] Antibody Concentration and Receptor Saturation

[0176] Serum samples were collected from patients at each visit and analyzed quantitatively for antibodies directed specifically against natalizumab using an enzyme linked immunosorbent assay (ELISA). Natalizumab serum levels and receptor occupancy by natalizumab were also measured in a subgroup of 12 to 14 patients per treatment group before each infusion and at 2 hours, 24 hours, 1, 2, and 3 weeks after the first and last infusions.

[0177] Serum antibody concentrations were determined using an ELISA assay. In brief, a 2.0 µg/mL solution of a capture antibody that binds specifically to natalizumab was prepared in a solution containing sodium bicarbonate to pH 8.3. 100 µL of the antibody solution was added to each well of a Costar 96-well microtiter plate. The plate was covered with plate sealing tape and incubated at ambient temperature for 12-26 hours. The plate was aspirated, 200 µL of blocking buffer (0.25% casein in PBS, pH 7.4) added to each well, and incubated for an additional hour at ambient temperature. Plates were then either desiccated and stored for later use, or washed once with 300 µL wash buffer (TBS, pH 7.5 containing 0.05% Tween-20). If plates were desiccated, they were rehydrated just prior to use by adding 300 µL wash buffer to each well and incubating for 1-2 minutes. Plates were aspirated and inverted onto tissue paper to absorb excess moisture.

[0178] Test samples were diluted in casein diluent prior to the ELISA (0.25% casein in PBS, with 0.05% Tween 20, pH 7.4). Typically, two or three dilutions of each sample are tested to ensure the natalizumab values were accurate. Dilution control samples were also prepared using known quantities of natalizumab to monitor the accuracy of the remaining steps.

[0179] 100 µL of reference standard, test sample, or dilution control sample was added to each well, and the plate incubated between 60-75 minutes at ambient temperature. The plates were washed three times with wash buffer and remaining moisture removed. 100 µL freshly diluted mouse anti-human IgG4-alkaline phosphatase conjugate was added to each well, and the plates were incubated for an additional 60 minutes at ambient temperature. Following incubation, plates were washed four times with wash buffer, and remaining moisture removed. 100 µL fluorescent substrate A was added using a calibrated multichannel pipetter, and the plates incubated 45-60 minutes at ambient temperature. The levels in each well were determined using a finax Fluorescence Microplate Reader using the SOFTmax Pro Version 1.3.1 protocol file conc102.ppr.

[0180] The results are as shown in FIGS. 3 and 4. As shown in these figures, levels of natalizumab decreased between dosings, and by month 7 or 8 (i.e., 2 to 3 months following the final dosing) the antibody was undetectable in the majority of patients.

[0181] As well as measuring serum antibody concentration, levels of receptor saturation, and specifically levels of VLA-4 saturation, were determined during the dosing study

using FACS analysis. Determination of VLA-4 saturation was determined by an indirect immunoassay using flow cytometry.

[0182] Approximately 1 mL of a blood sample from a patient was aliquoted into two 15 mL polypropylene tubes, and cold wash buffer (human serum [Scantibodies Laboratory, Inc. Part 3SH341] diluted to 3% in PBS) added to the 14 mL mark on the tube. The tubes are centrifuged at 2,200 rpm for 5 minutes at 10-15° C., and the liquid aspirated and discarded. The cell pellet is resuspended in cold wash buffer to a total of 1 mL. 500 µg/mL of a natalizumab stock reference standard is prepared in wash buffer. 20 µL of the diluted natalizumab stock reference was added to the first tube, and 20 µL wash buffer was added to the second tube. Both tubes were incubated on ice for 30 minutes in the dark. After incubation, the tubes were filled with wash buffer up to the 14 mL mark, and centrifuged at 2,200 rpm for 5 minutes in the cold. The supernatant was aspirated, and the wash step repeated a second time. Following the second wash, the cells were resuspended in wash buffer to the 1 mL mark.

[0183] 10 µL of R-Phycocrythrin (PE)-conjugated anti-human CDw49d antibody (Pharmingen, Cat. #31475x) was added to each tube and the tubes vortexed gently. The tubes were incubated at 2-8° C. for 10-15 minutes in the dark. Following incubation, 2 mL 1xlysing solution, pH 7.4, was added and each tube gently vortexed. The tubes were incubated on ice for 10-15 minutes at 2-8° C., centrifuged 5 minutes in the cold, and the supernatant aspirated from the cell pellet. The cell pellet was resuspended in 4 mL cold staining buffer, and the tubes centrifuged again at 2,200 rpm in the cold. The supernatant was then very carefully removed, 0.5 mL of a fixative solution (Ortho's Fixative pH 7.6) added, and the tubes immediately vortexed to ensure that cells were resuspended in the fixative. The tubes were covered with aluminum foil until FACS assay.

[0184] Each sample was then for VLA-4 receptor saturation in the samples with and without analyzed natalizumab using the FACS Calibur flow cytometer and CellQuest™ software. The CellQuest™ software allows acquisition and analysis of data from the flow cytometer.

[0185] Receptor saturation levels are shown in FIG. 5. The levels of receptor saturation for months 1-4 were determined prior to that month's dosing. The levels of receptor saturation produced by one month dosage intervals were consistently fairly high, and these chronic levels were sufficient to maintain a suppression of the pathological inflammation and associated physiological hallmarks of the disease. On average, the levels of saturation were maintained for a month at a mean of at least 67% and a median of at least 75%. These levels were sufficient for the suppression of brain lesions in the treated patients (see FIG. 1). The minimum level of saturation determined in the study preinfusion from month 2 to month 5 (and week 21 following administration at month 5) is particularly low as compared to the mean and median values due to a single patient with an antibody response to natalizumab.

[0186] As the receptor saturation levels dropped in the patients, so did the efficacy of the treatment. Mean receptor saturation levels of 42% and median receptor saturation levels of 41% receptor saturation in the treated population were not associated with suppression of brain lesions in the

patient population (See FIGS. 2 and 5), and thus a chronic receptor saturation level above this is necessary for effective suppression of pathological inflammation using agents such as alpha-4 inhibitors.

[0187] Safety and Tolerability

[0188] Repeated natalizumab treatment, at either the 3 or 6 mg/kg dose, appeared well tolerated by patients with MS over a six month treatment period. Similar numbers of patients from each group experienced treatment-emergent adverse events. Though not significant, certain adverse events occurred more commonly with natalizumab compared to placebo (Table 3). A sustained mild lymphocytosis was seen in the natalizumab arms over the six month treatment period.

TABLE 3

ADVERSE EVENTS REPORTED MORE COMMONLY IN NATALIZUMAB-TREATED PATIENTS VERSUS PLACEBO*			
	PLACEBO (71)	3 MG/KG (68)	6 MG/KG (74)
Total number of patients with adverse events	68 (96%)	62 (91%)	70 (95%)
Body as whole			
Infection	10 (14%)	14 (21%)	14 (19%)
Digestive system			
Flatulence	0	4 (6%)	0 (0%)
Nervous system			
Circumoral paresthesia	1 (1.0%)	5 (7%)	2 (3%)
Respiratory system			
Sinusitis	3 (4%)	7 (10%)	3 (4%)
Pharyngitis	8 (11%)	10 (15%)	15 (20%)
Skin and appendages			
Rash	4 (6%)	6 (9%)	8 (11%)
Urogenital system			
Infection	10 (14%)	14 (21%)	11 (15%)

*To be included in the Table, a difference of at least 5 percent in the incidence of adverse events was required between the placebo arm and one of the natalizumab arms.

[0189] There were no significant differences in the number of serious adverse events (SAEs) reported in the placebo and treatment arms (i.e., 7 placebo-treated patients reported 11 SAEs, 5 patients receiving natalizumab 3 mg/kg reported 5 SAEs, and 3 patients receiving natalizumab 6 mg/kg reported 4 SAEs). Of these, four were considered to be immune-mediated and related to study drug. There was one anaphylactic reaction with urticaria and bronchospasm in the 3 mg/kg group, which was rapidly reversed with antihistamines and steroid treatment. There were three reports of serum sickness, one in each group including placebo. Only one event was accompanied by a change in complement levels and all occurred at the same investigative site. Overall, these events complicated fewer than one in 250 infusions.

[0190] There were no differences in the number of patients that discontinued treatment due to an adverse event between groups (i.e., 3 in the placebo group, 4 in the 3 mg/kg group and 3 in the 6 mg/kg group). There was one death in the study secondary to pleural carcinomatosis complicated by hemothorax in a placebo patient.

[0191] Rate of antibody formation was also assessed. Overall, 15 natalizumab-treated patients (11 percent) developed anti-natalizumab antibodies: 13 during the treatment

period, and 2 during the post-treatment follow-up period. The clinical relevance, if any, of the presence of anti-natalizumab antibodies is not currently known.

[0192] Maximal serum concentrations of natalizumab were dose dependent and there was no significant accumulation observed with repeated dosing. The patients receiving natalizumab at 3 mg/kg exhibited greater than 80% saturation of the VLA-4 receptor during the treatment period; receptor occupancy was higher (approximately 90%) and more prolonged in patients receiving natalizumab 6 mg/kg.

[0193] Post-Treatment Follow-up: Months 6-12

[0194] The cumulative number of new enhancing lesions and active scans (months 9 and 12 combined) were similar in all three groups (Table 2). There was a trend to less activity in the 6 mg/kg group at month 9. There was no significant difference in the total number of reported clinical relapses between the three groups: 24 in the placebo group, 24 in the 3 mg/kg group, and 26 in the 6 mg/kg group or in the number of relapses as determined by the predefined objective criteria.

[0195] This study is the first to provide strong MRI and clinical evidence in humans that selective inhibition of alpha-4 integrin-mediated leukocyte adhesion and trafficking is an effective approach to the chronic treatment of MS. Both dose levels of the alpha-4 integrin-specific humanized monoclonal antibody natalizumab demonstrated highly statistically significant effects compared with placebo on suppression of new Gd-enhancing inflammatory brain lesions in patients with MS over the six month treatment period. A reduction of these lesions in natalizumab-treated patients was observed one month after the first infusion and was sustained throughout the treatment period. For both dose levels, the reduction was approximately 90 percent, an effect greater than the 50 to 70 percent reduction reported with beta-interferons (MS/MRI Analysis Group, 1995 *Neurology* 4: 1277-1285; Jacobs et al., 1996 *Ann. Neurol.* 39: 285-294; and PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) Study Group, 1998 *Lancet* 352: 1498-1504).

[0196] Moreover, the effects of natalizumab on MRI outcomes in this trial are supported by clinical observations. This study was not prospectively powered to show effects on clinical outcomes. Nonetheless, treatment with natalizumab resulted in a significant reduction in relapse rate and a perception of increased well-being among patients. When all reported clinical relapses are considered, both natalizumab groups experienced significantly fewer relapses than the placebo group during six months of treatment. A significant reduction in these episodes was also observed using predefined relapse criteria; a more stringent measure, because it requires a change in objective signs. The effect of natalizumab on relapses exceeds that of the currently approved treatments for MS, which display approximately 30 percent effect (MS/MRI Analysis Group, supra; Jacobs et al., supra; PRISMS Study Group, supra; Johnson et al., 1995 *Neurology* 45: 1268-76).

[0197] Importantly, no rebound effects on new MRI lesions or relapses were observed in the natalizumab groups after termination of treatment. Further, monthly infusions of natalizumab for six months were well tolerated, and associated with a safety profile similar to placebo and acceptable for chronic treatment of MS.

[0198] The results of this study provide further support for the role of alpha-4 integrin, and the immune cells that express it, in the pathogenesis of acute inflammatory brain lesions in patients with MS. The reduction in new Gd-enhancing lesions was evident after one month of treatment. This observation suggests natalizumab acts early in lesion development by preventing the appearance of new lesions.

[0199] In summary, natalizumab has demonstrated strong effects on clinically meaningful parameters in this placebo-controlled trial in patients with relapsing MS. Therapy was well tolerated during this six-month trial. The beneficial effects of natalizumab on the appearance of new inflammatory CNS lesions, the occurrence of clinical relapses and improvement in patient well-being observed in this study indicate the potential for observing effects on disability in the longer term studies currently in progress.

Example 2

Controlled Trial of Natalizumabin Crohn's Disease

[0200] Methods

[0201] In a double-blind, placebo-controlled trial, 248 patients with moderate to severely active Crohn's disease (CD) were randomized to receive two infusions of placebo or infusion of natalizumab at 3 mg/kg followed by placebo; or two infusions of natalizumab at 3 mg/kg or 6 mg/kg, at a 4-week interval. Outcome measures included the Crohn's Disease Activity Index (CDAI), health-related quality of life (QOL), and serum C-reactive protein levels.

[0202] Natalizumab increased the rates of clinical remission and clinical response, and improved QOL in patients with active CD, while demonstrating a safety profile acceptable for treatment of this disease.

[0203] Patient Population

[0204] After receiving approval from the local ethics committee, each center screened male and female patients of at least 18 years of age who had clinical evidence of moderate to severely active CD defined as a CDAI of at least 220 but less than or equal to 450. Of 311 patients screened, 248 were randomized at thirty-five study centers in Belgium, the Czech Republic, Denmark, Germany, Israel, the Netherlands, Sweden, and the United Kingdom from September 1999 to August 2000. All patients gave informed, written consent. Patients who received methotrexate, cyclosporin, or any investigational agents within 3 months were excluded; patients receiving azathioprine or 6-mercaptopurine were required to have been on a stable dose for at least 4 months. Other exclusions included prior antibody treatment; current use of oral prednisolone at a dose greater than 25 mg/day; current use of an elemental diet or parenteral nutrition; infectious or neoplastic diseases of the bowel; bowel surgery within 3 months; presence of a colostomy, ileostomy, or a colectostomy with ileorectal anastomosis; symptoms due mainly to the presence of fibrotic strictures; and clinical impression that the patient was likely in the near term to require emergency abdominal surgery.

[0205] Study Design and Randomization

[0206] Eligible patients were randomly assigned to one of four treatment regimens according to a computer-generated block randomization schedule. Each group received two

intravenous infusions spaced by a 4-week interval. The four treatment regimens were two infusions of placebo; one infusion of natalizumab at 3 mg/kg followed by placebo infusion; and two infusions of natalizumab at 3 or 6 mg/kg. The investigator, all other study personnel, and patients were blinded to treatment assignment.

[0207] Study Procedures and Endpoints

[0208] The primary efficacy endpoint was the proportion of patients in remission (CDAI < 150) at week 6. The CDAI incorporates eight related variables: number of liquid or very soft stools per day, severity of abdominal pain or cramping, general well-being, presence of extraintestinal manifestations of disease, presence of an abdominal mass, use of antidiarrheal drugs, hematocrit, and body weight (Best et al., 1976 *Gastroenterology* 70: 439-441; and Summers et al., 1979 *Gastroenterology* 77: 847-69). Scores less than 150 indicated remission; scores between 150 and 219 indicated mildly active disease, between 220 and 450 indicated moderately active disease, and scores greater than 450 indicated severe activity. Additional prospective endpoints were the proportion of patients demonstrating clinical response (i.e., at least 70 point reduction in CDAI), health-related quality of life, as measured by an inflammatory bowel disease specific questionnaire, the IBDQ (Irvine et al., 1994 *Gastroenterology* 106: 287-96), and serum levels of C-reactive protein.

[0209] Safety evaluations including the recording of adverse events and monitoring of clinical laboratories were conducted throughout the study. An independent safety data monitoring committee provided an additional level of monitoring. Serum samples were collected at each visit and analyzed for antibodies against natalizumab by an enzyme linked immunosorbent assay (ELISA).

[0210] Statistical Analysis

[0211] All efficacy analyses utilized the intent-to-treat (ITT) last observation carried forward (LOCF) population, which comprised all patients randomized (n=248). Patients using rescue medications were classified as treatment failures. The safety population (n=244) comprised those randomized and dosed. Four patients were not dosed due to ineligibility.

[0212] All statistical tests were two-sided and at the 5 percent level of statistical significance. Three pair-wise tests of each active treatment compared to placebo were performed. Remission and response rates were analyzed by the Cochran Mantel-Haenszel chi-squared test (general association) (Landis et al., 1978 *Int'l. Stat. Rev.* 46: 237-54) using country as strata. The effect of covariates on remission and response (yes or no) was analyzed at week 6 using logistic regression.

[0213] The sample size of 60 subjects per group was calculated to provide 80 percent power at a 5 percent significance level to detect a difference in response rates assuming a 40 percent response rate in the natalizumab groups and a 15 percent response rate in the placebo group.

[0214] Two-way analysis of variance models with fixed effects for country and treatment group were used to compare the mean CDAI decreases from base-line. Contrasts comparing each of the active treatment groups to the placebo group were tested.

[0215] C-reactive protein and IBDQ data were analyzed using the Wilcoxon-Mann-Whitney test to compare the change from base-line between each of the three active treatment groups and placebo. In a prospectively planned analysis, the C-reactive protein levels were compared for patients with a value above the upper limit of the normal range of 8 mg/l at base-line (week 0).

[0216] Results

[0217] Demographic characteristics, CDAI scores, site of disease, and medications were comparable among the groups at base-line (Table 4). At the time of the week 0 assessment, most patients were receiving other medications for CD including 5-ASA compounds (48 to 64 percent), oral steroids (46 to 63 percent), or azathioprine/6-mercaptopurine (18 to 37 percent) with or without other agents. Twenty-seven patients withdrew from the study prior to completing 12 weeks: 10 in the placebo group, and 6, 5, and 6 in the 3+0, 3+3, and 6+6 mg/kg natalizumab groups, respectively.

TABLE 4

DEMOGRAPHIC CHARACTERISTICS AND MEDICATIONS AT BASE-LINE				
CHARACTERISTIC	PLACEBO	3 + 0 MG/KG	3 + 3 MG/KG	6 + 6 MG/KG
Patients randomized	63	68	66	51
Mean age and range (yr)	34 (18-68)	36 (18-66)	36 (19-64)	35 (19-62)
Mean disease duration and range (yr)	8.9 (0.3-64.3)	8.4 (0.5-27.5)	8.1 (0.5-22)	7.8 (0.6-29)
Mean base-line CDAI and range*	300 (186-447)	288 (211-427)	298 (219-442)	296 (210-429)
Disease site:				
Ileal	15 (24%)	9 (13%)	17 (26%)	12 (24%)
Colonic	11 (17%)	16 (24%)	16 (24%)	16 (31%)
Ileocolonic	37 (59%)	43 (63%)	33 (50%)	23 (45%)
Gender: Female	33 (52%)	41 (60%)	36 (55%)	26 (51%)
Mean weight and range (kg)	68 (42-100)	66 (41-95)	64 (44-97)	69 (44-98)
Concomitant Medications				
No concomitant medications* for Crohn's disease	12 (19%)	11 (16%)	9 (14%)	5 (10%)
5 ASA compounds	30 (48%)	40 (59%)	42 (64%)	30 (59%)

TABLE 4-continued

DEMOGRAPHIC CHARACTERISTICS AND MEDICATIONS AT BASE-LINE				
CHARACTERISTIC	PLACEBO	3 + 0 MG/KG	3 + 3 MG/KG	6 + 6 MG/KG
Oral steroids	31 (49%)	31 (46%)	37 (56%)	32 (63%)
Azathioprine or 6-MP (±steroids)	2 (3%)	25 (37%)	17 (26%)	9 (18%)

* Eight of 248 patients had a CDAI score <220 at base-line. Five of these 8 patients were eligible (CDAI ≥ 220) on the basis of their screening hematocrit, which was the value available at randomization, but had scores <220 when subsequently recalculated using the base-line hematocrit. The CDAI scores for the other 3 patients were incorrectly calculated at the time of randomization.

† 5 ASAs, steroids, or immunosuppressants.

[0218] Clinical Responses, Remissions, and Mean CDAI Scores

[0219] The proportion of patients achieving a clinical response (i.e., at least a 70 point reduction in CDAI from base-line) was statistically significantly greater than placebo in all three natalizumab groups at weeks 4, 6, and 8 (Table 5 and FIG. 6), and this effect persisted through week 12 in the two groups that received 2 infusions of natalizumab. Trends for improvement in clinical response rates were observed as early as 2 weeks after the first treatment, and the 3+3 mg/kg natalizumab group demonstrated a statistically significant difference from placebo at this time point. Results for the decrease from base-line in mean CDAI score (Table 6) agree with the response rate findings.

TABLE 5

RATES OF REMISSION AND CLINICAL RESPONSE IN THE ITT POPULATION				
TIME POINT	PLACEBO (N = 63)	3 + 0 MG/KG NATALIZUMAB (N = 68)	3 + 3 MG/KG NATALIZUMAB (N = 66)	6 + 6 MG/KG NATALIZUMAB (N = 51)
REMISSION (CDAI <150) NO. OF PATIENTS (%)				
Week 2	6 (10)	10 (15)	13 (20)	6 (12)
P value		0.328	0.127	0.745
Week 4	9 (14)	21 (31)	19 (29)	15 (29)
P value		0.02	0.027	0.028
Week 6 (prim, endpoint)	17 (27)	20 (29)	29 (44)	16 (31)
P value		0.757	0.030	0.533
Week 8	10 (16)	19 (28)	27 (41)	22 (43)
P value		0.107	<0.001	<0.001
Week 12	17 (27)	19 (28)	28 (42)	21 (41)
P value		0.992	0.042	0.091
RESPONSE (≥70 POINT DROP) NO. OF PATIENTS (%)				
Week 2	19 (30)	31 (46)	36 (55)	22 (43)
P value		0.081	0.004	0.136
Week 4	18 (29)	32 (47)	41 (62)	27 (53)
P value		0.029	<0.001	0.006
Week 6	24 (38)	40 (59)	47 (71)	29 (57)
P value		0.022	<0.001	0.039
Week 8	22 (35)	38 (56)	44 (67)	28 (55)
P value		0.018	<0.001	0.028
Week 12	27 (43)	34 (50)	40 (61)	33 (65)
P value		0.503	0.033	0.018

[0220] Bold highlights the statistically significant results when compared to placebo.

TABLE 6

DECREASE FROM BASE-LINE IN MEAN CDAI SCORE				
TIME POINT	PLACEBO (N = 63)	3 + 0 MG/KG NATALIZUMAB (N = 68)	3 + 3 MG/KG NATALIZUMAB (N = 66)	6 + 6 MG/KG NATALIZUMAB (N = 51)
MEAN DECREASE IN CDAI SCORE FROM BASE-LINE (SD)				
Week 2	39.3 (73.5)	63.5 (74.8)	80.0 (68.9)	60.5 (68.3)
P value vs. placebo		0.061	0.001	0.103
Week 4	37.3 (88.8)	79.9 (89.5)	100.7 (76.4)	84.2 (90.5)
P value vs. placebo		0.004	<0.001	0.003
Week 6	49.3 (97.8)	81.5 (86.1)	119.4 (79.1)	97.2 (94)
P value vs. placebo		0.042	<0.001	0.004
Week 8	49.7 (99.5)	82.4 (87.2)	117.8 (90.7)	106.9 (102.9)
P value vs. placebo		0.053	<0.001	0.001
Week 12	63.1 (103.9)	70.1 (91.7)	119.2 (111)	112.5 (96.4)
P value vs. placebo		0.729	0.001	0.008

Decrease from baseline is defined as (week 0-week n)

SD = standard deviation

Bold highlights the statistically significant results when compared to placebo.

[0221] Four weeks after the first treatment, and before patients received the second treatment, all three natalizumab groups had a statistically significantly higher rate of remission compared to patients in the placebo group (Table 5 and FIG. 7). However, at week 6, the prospectively defined primary endpoint, the proportion of patients in clinical remission, was statistically significantly higher only in the 3+3 mg/kg natalizumab group compared to placebo. At week 8, both groups that received two infusions of natalizumab (either 3 or 6 mg/kg) demonstrated statistically significantly higher remission rates compared to placebo. At week 12, the 3+3 mg/kg natalizumab group continued to

were less likely to achieve remission. However, base-line CDAI did not predict the likelihood of response. All other variables analyzed were not significant predictors of remission or response.

[0224] Quality of Life

[0225] A statistically significant improvement in mean IBDQ scores was observed in all natalizumab treatment groups at week 6 compared to placebo. By week 12, only the treatment groups that received two infusions of natalizumab continued to have IBDQ scores that were significantly higher than the placebo group (Table 7).

TABLE 7

MEAN IBDQ SCORES				
TIME POINT	PLACEBO (N = 63)	3 + 0 MG/KG NATALIZUMAB (N = 68)	3 + 3 MG/KG NATALIZUMAB (N = 66)	6 + 6 MG/KG NATALIZUMAB (N = 51)
IBDQ SCORE, MEAN (RANGE)				
Week 0	130 (66-192)	130 (52-188)	136 (79-194)	123 (55-194)
Week 6	142 (61-219)	157 (81-221)	163 (99-211)	155 (67-224)
P value		0.008	<0.001	<0.001
Week 12	145 (61-217)	151 (81-221)	163 (86-221)	153 (64-215)
P value		0.486	0.021	0.014

demonstrate a statistically significant benefit over placebo for clinical remission, while the 6+6 mg/kg group demonstrated a strong trend for benefit over placebo for this outcome.

[0222] Predictors of Remission or Response

[0223] Using the results at week 6, an analysis was performed to identify base-line variables that may predict remission or clinical response. The variables examined included site of disease, duration of disease, base-line CDAI scores, concomitant use of oral steroids, concomitant use of azathioprine or 6-mercaptopurine, and extraintestinal symptoms. Base-line CDAI score was a significant predictor of remission ($P < 0.001$); patients with a higher base-line CDAI

[0226] Bold highlights improvements in the IBDQ score when compared to each treatment base-line.

[0227] C-Reactive Protein

[0228] Patients who had elevated levels of serum C-reactive protein at base-line received periodic assessments of those levels during the trial. Those who received two infusions of natalizumab exhibited a significant decline from base-line in mean serum levels of C-reactive protein compared to patients in the placebo group (FIG. 8). The improvement was maintained through week 12 in patients who received two infusions of 3 mg/kg natalizumab.

[0229] Safety and Tolerability

[0230] Natalizumab treatment at all dose levels was well tolerated throughout the 12-week study period. Similar numbers of patients from each group withdrew from the study for adverse events (i.e., 2 in the placebo group, 1 in the 3 mg/kg natalizumab group, 2 in the 3+3 mg/kg natalizumab group, and 3 in the 6+6 mg/kg natalizumab group). Overall, 31 patients reported a serious adverse event during the main study phase (i.e., 9 in the placebo group, 8 in the 3 mg/kg natalizumab group, 8 in the 3+3 mg/kg natalizumab group, and 6 in the 6+6 mg/kg natalizumab group). No serious adverse events were assessed as related to study drug and none were fatal; the majority were hospital admissions for complications or symptoms associated with CD. The numbers of patients experiencing treatment-emergent adverse events were similar in the various treatment groups: 52 (83 percent) in the placebo group, 51 (78 percent) in the 3 mg/kg natalizumab group, 57 (88 percent) in the 3+3 mg/kg natalizumab group, and 41 (80 percent) in the 6+6 mg/kg natalizumab group. Table 8 shows adverse events not related to CD with an incidence at least 5 percent greater in a natalizumab group compared to the placebo group. None of these various types of adverse events were statistically significantly different from placebo, and none are deemed to present an unacceptable safety profile for the use of natalizumab in patients with moderate to severe CD.

TABLE 8

ADVERSE EVENTS NOT RELATED TO CROHN'S DISEASE WITH >5% INCIDENCE OVER PLACEBO AND OTHER EVENTS OF INTEREST				
PREFERRED TERM	PLACEBO N = 63 (%)	3 + 0 N = 65	3 + 3 N = 65	6 + 6 N = 51
Chest pain	0 (0)	2 (3)	2 (3)	4 (8)
Conjunctivitis	0 (0)	2 (3)	5 (8)	0 (0)
Dizziness	0 (0)	6 (9)	3 (5)	0 (0)
Fever	1 (2)	4 (6)	3 (5)	4 (8)
Flu syndrome	6 (10)	9 (14)	7 (11)	10 (20)
Headache	20 (32)	19 (29)	27 (42)	14 (27)
Pain	4 (6)	4 (6)	4 (6)	11 (22)
Sinusitis	0 (0)	0 (0)	0 (0)	3 (6)
OTHER EVENTS:				
Infusion reaction*	0 (0)	0 (0)	1 (2)	1 (2)
Pts with anti-natalizumab antibodies	0 (0)	8 (13)	4 (6)	1 (2)

*Leading to interruption or cessation of infusion.

[0231] Anti-natalizumab antibodies were detected in 13 natalizumab-treated patients (7 percent) at week 12. Overall, patients with detectable anti-natalizumab antibodies were no more likely to experience an adverse event or serious adverse event than those who had no detectable anti-natalizumab antibodies.

[0232] Two patients experienced infusion reactions; in both, the event occurred during the second infusion. A patient in the 3+3 mg/kg natalizumab group experienced symptoms of mild itching and erythema, and was subsequently found to be positive for anti-natalizumab antibodies. A patient in the 6+6 mg/kg natalizumab group experienced symptoms of mild itching and coughing. These symptoms resolved without treatment, and the patient was subsequently found to be negative for detectable anti-natalizumab antibodies.

[0233] Although the prospectively defined primary efficacy endpoint (clinical remission defined as CDAI<150 at week 6) was statistically significantly superior to placebo only for the 3+3 mg/kg natalizumab group, significant benefit over placebo was observed for this outcome at 4 separate time points for the 3+3 mg/kg group (weeks 4, 6, 8, and 12) and two time points for the 6+6 mg/kg group (weeks 4 and 8, with a trend for benefit at week 12). Combined with the findings that the proportions of patients experiencing a clinical response (defined as at least a 70 point drop in CDAI from base-line) were significantly superior to placebo for all three natalizumab groups at the week 4, 6, and 8 time points, and at the week 12 time point for the two treatment groups that received two infusions of natalizumab, these data provide strong evidence for efficacy of natalizumab in the treatment of moderate to severely active CD. Further, the beneficial effects of natalizumab on clinical response and remission rates based on improvements in the CDAI are corroborated by significant improvements in health related QOL, measured by the IBDQ, and improvement in serum C-reactive protein, an acute phase reactant used to quantify generalized inflammation.

[0234] The dosage levels of receptor saturation in the CD trial are comparable to the dosages of the MS trial, and thus receptor saturation levels should be comparable in the CD trial. Receptor saturation levels in the CD trial are thus associated with decreased levels of inflammation, as demonstrated by C-reactive protein levels and an improvement in overall patient well-being, as demonstrated by the CDAI.

[0235] No serious safety concerns were identified for any of the natalizumab treatment groups. The percentage of patients developing detectable anti-natalizumab antibodies was low, and there were no serious adverse events associated with detectable anti-natalizumab antibodies.

[0236] The present study provides compelling evidence for the effectiveness and tolerability of alpha-4 integrin antagonism by natalizumab in the treatment of the clinical signs and symptoms of active moderate to severe CD. In addition, the evidence these data provide that alpha-4 integrin antagonism is an effective mechanism for treating CD raises the possibility that this modality may be more broadly applicable to the treatment of chronic autoimmune and inflammatory diseases.

Example 3

The Effect Of Natalizumab on Circulating
Activated Leucocytes in Active Inflammatory
Bowel Disease

[0237] The trafficking of leukocyte subsets is involved in the pathogenesis of inflammatory bowel disease (IBD). Because alpha-4 integrins are key mediators of leukocyte migration across the vascular endothelium, being that they are expressed on all leukocytes except neutrophils, the effects of a single 3 mg/kg natalizumab (Antegren®) infusion on basic circulating leukocyte subsets, natural killer (NK) cells and activated T cells in active inflammatory bowel disease patients (IBD). It had also previously been shown that a single 3 mg/kg infusion of natalizumab produced a sustained rise in circulating peripheral blood leukocytes in animals and healthy volunteers ("A six-month weekly intravenous toxicity study with Antegren™ in cyno-

molgous monkeys with a six-week recovery," *Athena Report* 1998, No. 723-013-098). However, it was unknown whether natalizumab could have differential effects on leukocyte subsets, given that all leukocytes except neutrophils express alpha-4 integrins.

[0238] Methods. Leucocytes extracted from peripheral blood of 30 Crohn's disease (CD; 18 natalizumab, 12 placebo) and 10 ulcerative colitis (UC; all received natalizumab—no placebo) patients pre-, 1-, 2-, 4-, 8- and 12-weeks post-infusion were analyzed by fluorescence-activated cell-sorter (FACS). Serum natalizumab levels and disease activity scores were measured at each time-point.

[0239] Significant changes compared to baseline ($p < 0.05$) and correlations were tested by Wilcoxon and Spearman tests respectively. Wilcoxon signed rank test for analysis of changes in leukocyte subsets were compared to baseline within treatment groups ($p < 0.05$ denotes significance). Spearman rank correlation tests were used to assess correlation between disease activity parameters, natalizumab levels and leukocyte subsets in those patients receiving natalizumab.

[0240] Venous blood was taken immediately prior to natalizumab/placebo infusion and again at one, two, four, eight and twelve weeks post-infusion. The Lymphoprep™ method (Nycomed, Denmark) was used to isolate the peripheral blood lymphocytes (PBLs) prior to analysis by multi-color fluorescence-activated cell sorter (FACS; Becton Dickinson, Oxford, UK) in conjunction with Consort 30 software (Amlot et al., 1996 *Clin. Exp. Immunol.* 105: 176-82). The percentages of PBLs expressing the following markers were measured using FACS analysis: CD19 (B cell), TCRαβ (T cell), CD3 (pan-T cell), TCRγδ (T cell), CD4 (helper/Th-1 T cell), CD8 (cytotoxic/suppressor T cell) and CD16 (NK cell).

[0241] The percentages of TCRαβ cells expressing the activation antigens CD38, CD25 (a chain of interleukin-2 receptor), CD26, CD69 and HLA-DR were measured, in addition to naive (CD45RA) and memory (CD45RO) T cell subsets and "NK-T cells" (CD57+/CD3+). The percentage of cytotoxic/suppressor T cells (CD8+) expressing the activation antigens CD28 and HLA-DR were also measured.

[0242] Results. Eosinophil, monocyte, B and T cell counts were all significantly raised for ≥ 1 week post-natalizumab. Total lymphocyte counts, both B and T cells, were significantly increased as compared to baseline levels. Neutrophil and basophil counts were unchanged. T cells expressing the activation markers CD25, CD26, HLA-DR, CD8DR, CD8, CD28, CD45RO and CD45RA were significantly raised

compared to baseline at ≥ 4 and 1 week in UC and CD patients respectively. CD38+ and CD69+ T cells were raised at ≥ 1 week in UC patients only. NK cells were unchanged post-infusion in all patients and NK-type T cells (CD57+) were raised at 1 week in CD patients only. No significant changes in gamma-delta (γδ) T cells were found in CD/UC patients. Changes in T cell subsets did not correlate with disease activity or serum natalizumab levels. The leukocyte changes found post-natalizumab were not detected following placebo.

[0243] The lymphocyte counts remained elevated at 4 weeks post-infusion in both Crohn's disease patients ($p = 0.002$) and in those patients with ulcerative colitis ($p = 0.02$), before returning to pre-treatment values at week 8 (Gordon et al., 2002 *Aliment. Pharm. & Ther.* 16: 699-706; and Gordon et al., 2001 *Gastroenterology* 121: 268-74).

[0244] The effect of natalizumab on circulating eosinophils and monocytes are shown in FIGS. 9A and 9B. The neutrophil and basophil counts were observed to remain unchanged in all the patient groups studied. FIGS. 10 and 11 demonstrate the effects of natalizumab administration on specific circulating T cell subsets and natural killer cells in Crohn's disease and ulcerative colitis patients. The error bars denote standards of deviation in each chart.

[0245] No significant changes in basic leukocyte subsets were detected following placebo infusion. Administration of the placebo to the 11 placebo patients is shown in Table 9. Some changes in lymphocytes expressing activation antigens were detected at isolated time points only in Crohn's patients (Table 10).

TABLE 9

LEUKOCYTE SUBSETS IN PLACEBO-TREATED CROHN'S DISEASE PATIENTS

Subset Cells	Week 0		Week 1		Week 2		Week 4		Week 8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
×10 ⁶ /mL										
Lymphocytes	.60	.33	.55	.36	.82	.67	.48	.29	.51	.31
Neutrophils	.21	.75	.18	.48	.86	.36	.61	.26	.91	.15
Eosinophils	.19	.16	.17	.13	.15	.13	.20	.12	.14	.10
Basophils	.19	.30	.14	.09	.09	.07	.09	.03	.08	.05
Monocytes	.60	.33	.55	.36	.82	.67	.48	.29	.51	.31

Mean(SD) leukocyte subset values post-placebo; no significant differences in any group compared to baseline (week 0) values.

[0246]

TABLE 10

T CELL AND NK MARKERS IN PLACEBO-TREATED CROHN'S DISEASE PATIENTS

Subset Cells	Week 0		Week 1		Week 2		Week 4		Week 8		Week 12	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
×10 ⁶ /mL												
Lymphocytes	.36	.57	.12	.57	.83	.08	.34	.98	.28	.74	.62	.84
TCRαβ+	.99	.53	.81	.56	.28	.88	.98	.84	.95	.79	.17	.81
CD25+	.18	.11	.12	.07	.27	.22	.17	.18	.15	.22	.17	.17
CD26+	.49	.32	.38	.27	.80	.61	.54	.49	.54	.55	.58	.43

TABLE 10-continued

T CELL AND NK MARKERS IN PLACEBO-TREATED CROHN'S DISEASE PATIENTS													
Subset Cells	Week 0		Week 1		Week 2		Week 4		Week 8		Week 12		SD
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
CD45RA+	.74	.41	.57	.39	.95	.69	.68	.59	.68	.57	.84	.56	
CD45RO+	.42	.20	.31	.18	.50	.27	.39	.24	.42	.35	.50	.33	
CD38+	.42	.29	.31	.23	.46	.38	.35	.27	.33	.25	.40	.25	
CD69+	.14	.20	.67	.71	.13	.12	.05	.04	.12	.22	.10	.08	
HLA-DR+	.19	.10	.15	.08	.25	.20	.16	.11	.17	.10	.22	.16	
CD8+	.11	.09	.09	.06	.16	.15	.09	.07	.08	.05	.12	.11	
CD8+CD28+	.21	.11	.16	.11	.30	.20	.22	.21	.20	.18	.23	.14	
CD8+DR+	.11	.09	.09	.06	.16	.15	.09	.07	.08	.05	.12	.11	
CD57+CD3+	.08	.06	.06	.05	.10	.10	.07	.04	.07	.05	.10	.15	
CD16+CD3-	.11	.09	.12	.09	.16	.18	.09	.04	.12	.12	.17	.19	
TCR $\gamma\delta$ +	.11	.16	.08	.09	.18	.28	.10	.13	.09	.09	.11	.10	
KappaMAb+	.02	.02	.03	.05	.05	.08	.07	.09	.03	.04	.10	.01	

Columns show mean counts (SD) of T cell subsets and NK-type cells of Crohn's study placebo patients compared with baseline values (Wilcoxon signed rank test; $p < 0.05$). Significant differences compared to baseline are shown in bold. Standard deviations for each mean are shown in italic numbers.

[0247] There was no significant correlation between the total lymphocyte counts and disease activity score in either Crohn's disease or ulcerative colitis patients (Tables 11 and 12), nor were any significant correlations found between individual lymphocyte subsets and disease activity. No significant correlation was detected between serum natalizumab levels and changes in leukocyte subsets at one, two or four weeks post-infusion. Natalizumab was undetectable in almost all patients at eight weeks. Thus correlations were not calculated for this time point.

TABLE 11

SPEARMAN R VALUES COMPARING LYMPHOCYTE SUBSETS AND DISEASE ACTIVITY						
Subsets	Crohn's disease			Ulcerative colitis		
	Week 1	Week 2	Week 4	Week 1	Week 2	Week 4
Lymphocytes	0.16	-0.37	-0.01	-0.63	-0.19	0.03
TCR $\alpha\beta$	0.26	-0.29	0.05	-0.59	-0.39	0
CD25	0.44	0.07	0.13	-0.39	-0.46	0.17
CD26	0.31	-0.22	-0.11	-0.39	-0.29	0.10
CD45RA	0.29	-0.25	0.19	-0.54	-0.39	0.51
CD45RO	0.32	-0.27	0.34	0.18	-0.17	-0.05
CD38	0.31	-0.08	0.01	-0.57	0.03	-0.05
CD69	-0.32	0.12	0.12	-0.15	0.28	0.12
HLA-DR	0.19	-0.05	-0.01	0.12	-0.13	0.15
CD8CD28	0.01	-0.17	-0.13	-0.18	-0.04	-0.17
CD8DR	0.11	-0.19	-0.18	-0.1	0.07	0.24
CD57	0.19	-0.08	-0.04	0.21	-0.15	0.15
CD16	-0.08	-0.3	-0.15	-0.19	0.1	-0.32
TCR $\gamma\delta$	0.34	0.14	-0.31	-0.63	-0.47	-0.66
Kappa MAb	-0.11	-0.02	-0.2	0.05	-0.27	0.19

No significant correlation found between lymphocyte subset counts and CDAI (Crohn's disease patients) or Powell-Tuck score (ulcerative colitis).

[0248]

TABLE 12

SPEARMAN R VALUES COMPARING LYMPHOCYTE SUBSETS WITH SERUM NATALIZUMAB						
Subsets	Crohn's disease			Ulcerative colitis		
	Week 1	Week 2	Week 4	Week 1	Week 2	Week 4
Lymphocytes	0.03	0.49	0.64	0.1	-0.02	0.18
TCR $\alpha\beta$	-0.11	0.35	0.51	-0.04	0.28	-0.11
CD25	-0.09	0.03	0.26	0.25	-0.07	-0.25
CD26	-0.07	0.28	0.61	0.20	0.22	-0.14
CD45RA	0.19	0.29	0.62	-0.12	-0.25	-0.32
CD45RO	-0.16	0.32	0.34	0.37	0.08	0.39
CD38	0.06	0.18	-0.34	-0.01	-0.45	-0.43
CD69	0.02	0.26	0.57	0.2	-0.22	0.14
HLA-DR	0.05	0.13	-0.25	0.08	0.27	-0.07
CD8CD28	-0.18	0.16	0.41	0.25	0.07	0.32
CD8DR	0.4	0.16	0.004	0.08	0.3	-0.18
CD57	0.1	-0.06	-0.31	-0.08	0.03	0
CD16	0.22	0.19	-0.5	0.13	0.37	0.25
TCR $\gamma\delta$	0.002	-0.16	-0.2	0.21	0.61	0.6
Kappa MAb	-0.07	-0.27	0.29	0.41	0.45	-0.41

No significant correlation between disease activity and lymphocyte subsets, except for total lymphocyte counts at week 4 ($p = 0.04$).

[0249] Based on the above data, a single 3 mg/kg natalizumab infusion produced increased circulating levels of most, but not all, leukocyte subsets in patients with active IBD. Circulating eosinophil, monocyte and lymphocyte counts were significantly elevated above baseline values for at least four weeks post-infusion in most patients. A wide range of circulating T cell subsets were significantly increased above pretreatment values, particularly those expressing activation antigens. However, NK cell counts (CD16⁺/CD3⁻) were not affected by natalizumab, and CD57⁺ T cells were affected to a much lesser extent by natalizumab than other T cell subsets. Lymphocytes expressing the $\gamma\delta$ T cell receptor were also not affected by natalizumab, suggesting that alpha-4 integrins are either not expressed, or are expressed at a lower level on these cells.

[0250] Thus, in active IBD patients, natalizumab may limit trafficking and maintain in circulation many leukocyte and activated lymphocyte subsets. The NK cells, $\gamma\delta$ cells, neutrophils and basophils appear unaffected by the administration of natalizumab, which may suggest that they are less important mediators in the trafficking of these cell types.

[0251] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the invention.

[0252] All references cited herein are herein incorporated by reference in their entirety for all purposes.

We claim:

1. A method of chronically reducing pathological inflammation in a patient in need thereof comprising chronically administering an agent to the patient that inhibits alpha-4 integrin or inhibits a dimer comprising alpha-4 integrin in a therapeutically effective amount.

2. The method of claim 1, wherein the chronic administration is for a period of at least 6 months.

3. The method of claim 2, wherein the chronic administration is for a period of at least 12 months.

4. The method of claim 1, wherein the agent is administered repeatedly to the patient to bind to alpha-4 integrin or a dimer comprising alpha-4 integrin, and wherein the administration maintains alpha-4 integrin receptor saturation at a level sufficient to chronically suppress pathological inflammation in the patient.

5. The method of claim 4, wherein the agent is administered repeatedly to the patient such that alpha-4 integrin receptor saturation is about at least 65% to about 100% in the patient.

6. The method of claim 5, wherein the saturation is at least 75%.

7. The method of claim 5, wherein the saturation is at least 80%.

8. The method of claim 1, wherein the agent binds to alpha-4 integrin dimers.

9. The method of claim 1, wherein the agent is a monoclonal antibody or an immunologically active fragment thereof.

10. The method of claim 9, wherein the monoclonal antibody is natalizumab.

11. The method of claim 1, wherein the alpha-4 integrin dimer is alpha-4 beta-1.

12. The method of claim 4, wherein the agent is administered in amount sufficient to saturate at least one alpha-4 integrin dimer receptor thereby inhibiting pathological inflammation.

13. The method of claim 12, wherein the dimer receptors are alpha-4 beta-1 or alpha-4 beta-7, and the pathological inflammation is caused by multiple sclerosis.

14. The method of claim 12, wherein the dimers receptors are alpha-4 beta-1 or alpha-4 beta-7, and the pathological inflammation is caused by an inflammatory disease of the gastrointestinal tract.

15. The method of claim 14, wherein the inflammatory disease of the gastrointestinal tract is Crohn's Disease, ulcerative colitis or inflammatory bowel disease.

16. The method of claim 1, wherein the pathological inflammation is caused by an inflammatory disease of the gastrointestinal tract.

17. The method of claim 16, wherein the inflammatory disease of the gastrointestinal tract is Crohn's disease, ulcerative colitis, or inflammatory bowel disease.

18. The method of claim 1, wherein the pathological inflammation is caused by multiple sclerosis.

19. A method of determining the efficacy of a chronic administration regime for treating pathological inflammation in a subject, wherein the pathological inflammation is modulated by an alpha-4 integrin comprising measuring saturation of the alpha-4 integrin or a dimer comprising alpha-4 integrin.

20. The method of claim 19, wherein the alpha-4 integrin dimer is alpha-4 beta-1 or alpha-4 beta-7.

21. The method of claim 19, wherein the pathological inflammation is caused by Crohn's Disease, and measurement of the C-reactive protein and/or CDAI in the patient measures saturation of alpha-4 integrin.

22. The method of claim 19, wherein the pathological inflammation is caused by multiple sclerosis.

23. The method of claim 1, wherein the pathological inflammation is an inflammatory disease of the gastrointestinal tract and comprises chronically administering a therapeutically effective dose of natalizumab or an immunologically active fragment thereof to a patient in need thereof sufficient to treat or ameliorate the inflammatory disease of the gastrointestinal tract in the patient.

24. The method of claim 23, wherein the disease of the gastrointestinal tract is inflammatory bowel disease, Crohn's disease or ulcerative colitis.

25. The method of claim 23, wherein natalizumab is administered by infusion every four weeks for at least 6 months in an amount of about 1 mg/kg patient to about 20 mg/kg patient.

26. The method of claim 25, wherein the infusions are administered for at least 12 months.

27. The method of claim 1, wherein the pathological inflammation is multiple sclerosis and comprises chronically administering a therapeutically effective dose of natalizumab or an immunologically active fragment thereof, to a patient in need thereof sufficient to relieve symptoms of multiple sclerosis.

28. The method of claim 27, wherein natalizumab is administered by infusion every four weeks for at least 6 months in an amount of about 1 mg/kg patient to about 20 mg/kg patient.

29. The method of claim 28, wherein the infusions are administered for at least 12 months.

30. A composition for chronic treatment of pathological inflammation in a patient comprising an agent in an amount sufficient to relieve symptoms of the pathological inflammation in the patient in need thereof.

31. The composition of claim 30, wherein the agent inhibits alpha-4 integrin activity and/or alpha-4 integrin dimer activity.

32. The composition of claim 30, wherein the composition further comprises a stabilizer, a carrier and/or an excipient.

33. The composition of claim 30, wherein the agent is natalizumab.

34. The composition of claim 33, wherein natalizumab is formulated for intravenous infusion in the amount of about 1 mg/kg patient to about 20 mg/kg patient for administration every 4 weeks for at least 6 months.

35. The composition of claim 33, wherein the pathological inflammation is caused by multiple sclerosis.

36. The composition of claim 33, wherein the pathological inflammation is caused by an inflammatory disease of the gastrointestinal tract.

37. The composition of claim 36, wherein the inflammatory disease of the gastrointestinal tract is Crohn's disease, ulcerative colitis or inflammatory bowel disease.

38. A combination therapy for the chronic treatment of pathological inflammation in a patient, wherein the combination therapy comprises a composition of claim 30 and a compound that ameliorates the pathological inflammation.

39. The combination therapy of claim 38, wherein the pathological inflammation is caused by an inflammatory disease of the gastrointestinal tract.

40. The combination therapy of claim 39, wherein the inflammatory disease of the gastrointestinal tract is ulcerative colitis, inflammatory bowel disease or Crohn's disease, and the compound is a 5-aminosalicylate, a glucocorticoid, a thioguanine derivative, methotrexate, a cyclosporine, a monoclonal antibody that binds to TNF, or an antibiotic.

41. The combination therapy of claim 38, wherein the pathological inflammation is caused by multiple sclerosis.

42. The combination therapy of claim 41, wherein the compound is infliximab, interferon beta-1b, interferon beta-1a, copaxone®, a non-steroidal anti-inflammatory drug, or Novantrone®.

* * * * *

ANEXO 2

Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites

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SUMMARY

Unlike other immunoglobulin G (IgG) subclasses, IgG4 antibodies in plasma have been reported to be functionally monovalent. In this paper we show that the apparent monovalency of circulating IgG4 is caused by asymmetry of plasma IgG4. A large fraction of plasma IgG4 molecules have two different antigen-binding sites, resulting in bispecificity. Sera from patients with IgG4 antibodies to both house dust mite and grass pollen induced cross-linking of Sepharose-bound grass pollen antigen to radiolabelled house dust mite allergen *Der p* 1. This bispecific binding activity was not observed in sera with IgG4 antibodies to either grass pollen or house dust mite exclusively. Depletion of IgG4 antibodies resulted in disappearance of the bispecific activity. By size exclusion chromatography we excluded the possibility that bispecific activity was caused by aggregation of IgG4 antibodies. These results indicate that circulating (polyclonal) IgG4 antibodies have two different antigen-binding sites and therefore are functionally monovalent antibodies.

INTRODUCTION

The interaction of an antibody with an antigen initiates several immunological responses. The consequences of this antibody–antigen interaction are determined by the isotype of the antibody (mediating through the interaction with immunoglobulin receptors) and by the valency of the antibody–antigen interaction. Bivalency of an immunoglobulin G (IgG) antibody is an important aspect of most effector functions.

Human IgG4 antibody is unable to precipitate purified antigens.^{1,2} This inability to precipitate allergen was not due to a difference in affinity of the IgG4 antibodies, but was caused by the inability of IgG4 antibodies to cross-link two antigens: human serum IgG4 antibodies are functionally monovalent.^{1,2} In addition, IgG4 antibodies react poorly with Fc-receptors compared to the IgG1 antibody and do not efficiently activate complement.³ Nevertheless, in some individuals chimeric IgG4 antibody was shown to be as potent as IgG1 in antibody-dependent cell-mediated cytotoxicity.⁴ This unexpected effect, presumably due to a polymorphism, was also seen in an *in vivo* mouse model of immunotherapy.⁵

Generally, however, IgG4 antibodies are considered to be less efficient in inducing inflammatory responses and may even inhibit the inflammatory effects of other antibodies.^{6–11} Prolonged antigenic stimulation with high doses of soluble

protein antigen preferentially induces IgG4 antibodies. IgG4-dominated responses have been reported in allergic patients receiving immunotherapy,^{1,12,13} in beekeepers,¹ in patients with chronic parasitic infections, such as schistosomiasis and filariasis,^{8–11} and in haemophiliac patients receiving factor VIII.¹⁴ IgG4-mediated inhibition of immune inflammation may be advantageous in some instances, but may be undesirable in infections.

In this report we compared circulating and chimeric IgG4 antibodies. Our results indicate that the apparent monovalency of naturally occurring IgG4 antibodies is due to bispecificity.

MATERIALS AND METHODS

Monoclonal chimeric antibodies and sera

The mouse/human chimeric monoclonal IgG4 antibody (hIgG4-Dp2A) has been described previously.¹⁵ This chimeric antibody is directed against the house dust mite allergen *Der p* II and originates from the mouse hybridoma cell line 2B12.¹⁶ The rabbit hyperimmune antiserum to *Der p* I has been described elsewhere.¹⁷

The sera and plasma samples used were obtained from the Allergy Diagnostics Department (CLB, Amsterdam, the Netherlands). Nine sera were used in the heterologous binding assay (described below), plasma #741B (obtained by plasmapheresis) and serum #741 were derived from the same patient: plasma #178 (obtained by plasmapheresis) was used in the valency tests. This plasma contained 1400 ng of *Der p* II-specific IgG4 antibodies/ml as was determined by the method described previously.¹⁵ The IgG4 antibodies of this plasma were affinity purified using monoclonal antibody

Received 20 August 1998; revised 26 March 1999; accepted 17 April 1999.

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MH164-4M (CLB) coupled to CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden).

Radioimmunoassays (RIA)

Mite-extract. *Dermatophagoides pteronyssinus* mites were obtained from Commonwealth Serum Laboratories (Melbourne, Australia). 1.5 g of mites were extracted in phosphate-buffered saline (PBS; 3% w/v) supplemented with Tween-20 (0.1% v/v) and sodium azide (0.1% w/v) for 4 hr. After filtration (black ribbon filter, Schleicher & Schüll, Dassel, Germany), the extract was coupled to 10 g CNBr-activated Sepharose 4B (Pharmacia). Per test, 1.5 mg Sepharose in 250 µl was used, equivalent to 170 KU (0.17 µg) *Der p* II/test. This was also the optimal condition for the two-site assay described below.

Grass pollen. *Phleum pratense* and *Dactylis glomerata*, obtained from Diephuis Laboratories (Groningen, the Netherlands) were extracted as previously described¹⁸ and coupled to CNBr-activated Sepharose. Per test 1 mg Sepharose was used.

Iodination of allergens

Recombinant *Der p* II was used (kindly supplied by G. A. Mueller¹⁹). *Der p* II was purified by affinity chromatography using monoclonal antibody αDpX.¹⁷

The *Der p* II was iodinated by the chloramine-T method with carrier free ¹²⁵I (Amersham Life Science, Amersham, UK) as was described previously¹ with minor modifications, necessary for iodinating *Der p* II (20 µg in 100 µl PBS): 1-propanol was added to a final concentration of 30% (v/v) prior to labelling. The labelling was performed with 1 mCi of ¹²⁵I. Free label was separated from ¹²⁵I-labelled *Der p* II by dialysis against PBS containing 0.01% (w/v) PEG-4000. *Der p* I, affinity purified with anti-P1¹⁷ from *Dermatophagoides pteronyssinus* mite extract was labelled by the standard chloramine-T method.¹

Two-site assay: testing the valency of the antibodies

To test whether serum or chimeric antibodies were able to cross-link radiolabelled antigen to immunosorbent bound antigen, a two-site assay was performed. In this assay the amount of capturing antigen coupled to Sepharose has to be optimized in order to avoid bivalent binding of antibodies to adjacent antigens on the immunosorbent. To determine this, the amount of mite extract used for coupling to CNBr-activated Sepharose was titrated.

Sepharose-coupled mite-extract (described above) was incubated with 50 µl serum or chimeric antibody (1–20 ng antibody per test) in a total volume of 300 µl in PBS-AT (PBS supplemented with 0.3% bovine serum albumin, 0.1% Tween-20, 0.05% (w/v) NaN₃). The ability to cross-link was examined by addition of ¹²⁵I-labelled *Der p* II (in the valency tests) or *Der p* I (in the heterologous cross-linking assay).

Antigen-binding test

For the antigen-binding test 10 µl serum (or serum dilutions) or antibody (1–20 ng antibody/test) was incubated with 1 mg Protein A-Sepharose (Pharmacia) in 500 µl PBS-AT together with ¹²⁵I-labelled antigen. The results were expressed as the percentage of radioactivity bound relative to the total radioactivity added.

Heterologous cross-linking assay

Sera were tested for bispecific binding activity in the heterologous cross-linking assay. Fifty microlitres of serum (or serum dilutions) were incubated with grass pollen-Sepharose (1 mg/test) in a total volume of 300 µl in PBS-AT. Antibodies bound were detected using radiolabelled mite-allergen *Der p* I. The results were expressed as the percentage of radioactivity bound relative to the total radioactivity added.

Size-exclusion chromatography

Serum #741 (200 µl) was applied to a ACA34 column (Ultrogel LKB, Villeneuve-la-Garenne, France), 1.5-cm diameter and 35-cm length, 62-ml material, equilibrated in PBS-AT. Fractions of 450 µl were collected and analysed for total IgE (180 000 MW), *Der p* I-specific IgG (tested in the antigen-binding test for IgG) and for grass pollen/*Der p* I-bispecific binding activity (tested in the heterologous cross-linking assay). The void volume was determined using Dextran Blue 2000 (Pharmacia). The results were expressed as percentage of starting material.

IgG4 and IgE depletion

Serum #741 was depleted for IgG4 and IgE antibodies. Anti-IgG4 monoclonal antibody MH164-4M (CLB), 10 mg, was coupled to 1 g CNBr-activated Sepharose (Pharmacia). For the depletion of IgE antibodies polyclonal sheep antihuman IgE serum coupled to Sepharose (10 ml antiserum was coupled to 20 g CNBr-activated Sepharose) was used. As a control for the depletion experiment, serum was incubated with CNBr-activated Sepharose that had been quenched by the addition of glycine. For depletion of 40 µl serum #741, 16 mg anti-IgG4, anti-IgE, or inactivated CNBr-activated Sepharose was used. The depletion was performed by overnight incubation (end over end at room temperature) in a final volume of 500 µl PBS-AT.

RESULTS

Valency of natural and chimeric IgG4 antibodies directed to the house dust mite allergen *Der p* II

The amount of *Der p* II-specific IgG antibodies (or, more accurately, IgG₁₂₄ = IgG1 + IgG2 + IgG4 antibodies) in plasma #178 was determined in the antigen-binding test for *Der p* II, using as solid phase either Sepharose-coupled Staphylococcal protein A or Sepharose-coupled anti-IgG4; chimeric IgG4 antibody was used as a reference (Fig. 1). From the data presented in Fig. 1 the contribution of IgG4 antibody relative to IgG₁₂₄ antibody in this serum can be calculated to be 56%. Next, the bivalent reactivity of naturally occurring IgG4 antibodies (purified from plasma #178) was compared to the bivalent reactivity of the mouse/human chimeric IgG4 monoclonal antibody directed to the house dust mite allergen *Der p* II. Bivalent reactivity was measured as the ability to cross-link radiolabelled *Der p* II and Sepharose-bound *Der p* II (Fig. 2). Chimeric IgG4 induced cross-linking of radiolabelled *Der p* II to Sepharose-bound *Der p* II. In contrast, neither plasma #178 nor affinity purified plasma #178-derived IgG4 antibodies induced significant binding in this assay. Chimeric IgG1 and IgG4 antibodies were equally effective in cross-linking (not shown). These observations confirmed that naturally occurring (polyclonal) IgG4 antibodies behave as mono-

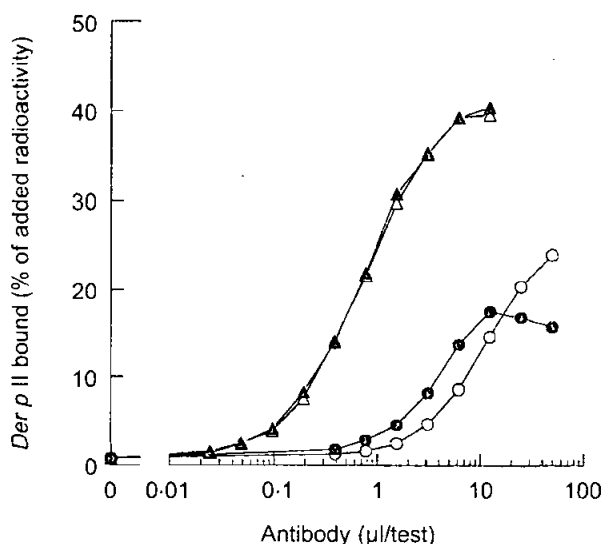


Figure 1. The relative contribution of IgG4 to the total IgG_{1,24} antibody activity with respect to the binding of ¹²⁵I-labelled *Der p* II of plasma #178 was deduced from the comparison between Sepharose-coupled anti-IgG4 (○) and Sepharose-coupled Staphylococcal Protein A (●); the results obtained with the chimeric IgG4 antibody, 17 µg/ml, are also shown (anti-IgG4, △, Protein A, ▲).

valent reagents towards purified *Der p* II in contrast to the bivalent behaviour of chimeric IgG4 antibodies.

Allergen-specific immunotherapy results in bispecific immunoglobulins

Sera from patients receiving therapeutic injections with both house dust mite and grass pollen extract were tested in a heterologous cross-linking assay. In this heterologous cross-linking assay the binding of purified radiolabelled house dust mite allergen *Der p* I to Sepharose-coupled grass pollen was measured (Table 1). All sera with IgG4 antibodies to both house dust mite allergen *Der p* I and to grass pollen allergen were able to cross-link mite-allergen *Der p* I to Sepharose-coupled grass pollen; binding ranging from 4.6 to 34.1% of added radioactivity. Sera of patients with IgG (IgG4) antibodies directed exclusively to house dust mite (represented by *Der p* I) or grass pollen (represented by *Lol p* I), were negative in this test (less than 2% binding of the amount of radioactivity added; the binding was indistinguishable from the binding of *Der p* I to control Sepharose to which an irrelevant ligand, glycine, had been coupled).

A fresh plasma sample from the patient that donated serum #741 was obtained, coded #741B. This sample showed similar results: 44.2% binding to Sepharose-coupled grass pollen and 0.8% binding to control Sepharose (binding expressed as percentage of labelled *Der p* I added).

To test whether a *Der p* I contamination of the allergosorbent (Sepharose-coupled grass pollen) was the cause of the bispecific binding of these sera, hyperimmune rabbit antiserum to *Der p* I was incubated with Sepharose-coupled grass pollen (Fig. 3). Although *Der p* I-binding activity of this hyperimmune rabbit antiserum exceeded the binding of plasma #741B,

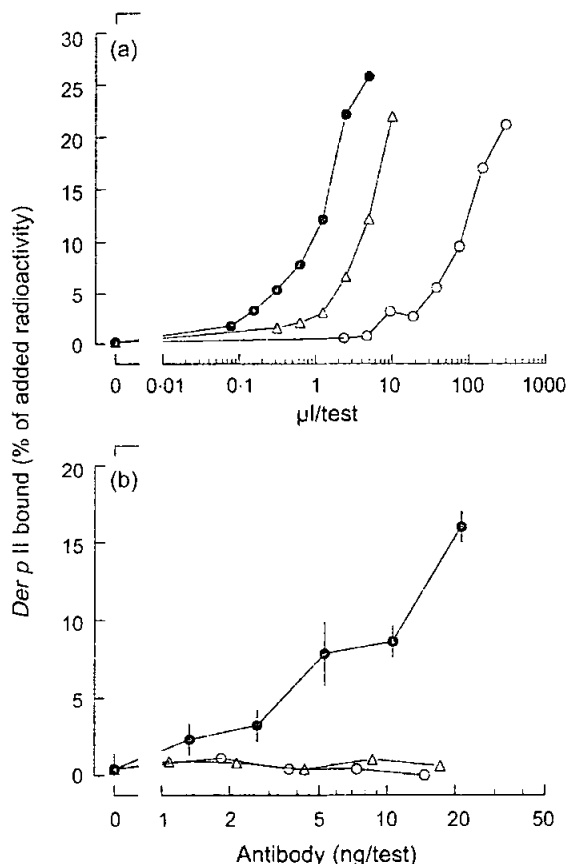


Figure 2. The functional valency of chimeric IgG4, plasma #178 and affinity-purified IgG4 from plasma #178. (a) Binding of ¹²⁵I-labelled *Der p* II to serial dilutions of chimeric IgG4 (17 µg/ml, ●), plasma #178 (△) and affinity purified IgG4 from plasma #178 (○) to Sepharose-coupled Staphylococcal Protein A were tested and used to determine the amount of *Der p* II-specific IgG_{1,24} in the plasma. (b) The cross-linking of Sepharose-coupled mite-extract to ¹²⁵I-labelled *Der p* II by chimeric IgG4 (●), plasma #178 (△) or affinity-purified IgG4 from plasma #178 (○) was expressed as the amount of radioactivity bound relative to the amount of radioactivity added.

the hyperimmune rabbit antiserum induced less than 1% binding of labelled *Der p* I to grass pollen-Sepharose.

Size fractionation of bispecific binding activity

Serum #741 was subjected to gel filtration to exclude the involvement of antibody aggregates. Size-exclusion chromatography was performed using ACA34. Fractions were tested for total IgE, *Der p* I-specific IgG and the ability to cross-link Sepharose-coupled grass pollen to *Der p* I (Fig. 4). *Der p* I-specific IgG and the bispecific binding activity were detected in the same fractions, eluting just behind IgE. This indicates that the heterologous cross-linking activity was due to antibodies with the size of monomeric IgG.

IgG subclass of bispecific binding activity: IgG4

To investigate whether IgG4 antibodies were responsible for the bispecific binding measured in the heterologous binding

Table 1. Sera from patients receiving immunotherapy treatment were tested in a heterologous binding test

Serum	Heterologous binding test*	Negative control*	<i>Der p 1</i>		<i>Lol p 1</i>	
			IgG†	IgG4†	IgG†	IgG4†
#701	0.7	0.4	0	0	89	54
#719	1.2	0.9	50	39	0	0
#748	1.3	NT	179	162	7	2
#831	4.6	0.4	92	11	52	16
#822	5.0	0.2	66	45	57	25
#766	7.9	0.5	64	11	152	16
#797	8.8	0.7	64	64	81	41
#614	10.7	0.4	58	47	96	50
#741	34.1	0.4	76	62	249	59

Bispecific binding, the binding to grass pollen–Sepharose and glycine-inactivated Sepharose (control Sepharose) of 1:10 diluted serum was detected by radiolabelled *Der p 1*; NT=not tested. *The results were expressed as the amount of radioactivity bound relative to the amount of radioactivity added. No correction for background binding was applied. †IgG and IgG4 antibodies to the house dust mite allergen *Der p 1* and the grass pollen allergen *Lol p 1* are expressed as arbitrary antibody units/ml (using a previously described method²⁷).

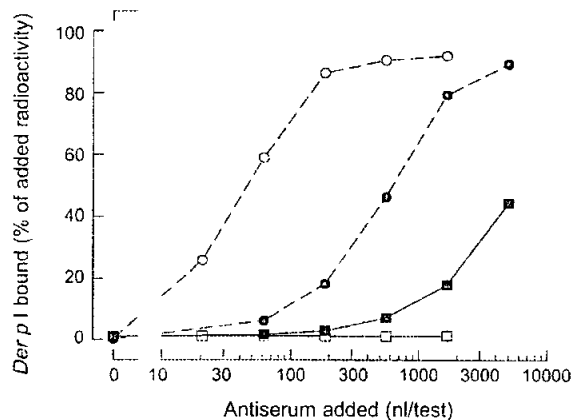


Figure 3. The bispecific binding is not caused by *Der p 1* contamination of the grass pollen–Sepharose. Serial dilutions of rabbit polyclonal antiserum to *Der p 1* (○, □) and plasma #741B (●, ■) were incubated with protein A–Sepharose (○, ●) or with grass pollen–Sepharose (□, ■). The binding was detected using radiolabelled *Der p 1*. The results were expressed as the amount of radioactivity bound relative to the amount of radioactivity added.

assay, serum #741 was depleted for its IgG4, using anti-IgG4 antibodies coupled to CNBr-activated Sepharose. As controls, the serum was also depleted for IgE antibodies (using anti-IgE–Sepharose) and was incubated with control Sepharose (glycine-inactivated Sepharose). After depletion, the three samples of manipulated serum #741 were tested in the antigen-binding test for *Der p 1* using protein A–Sepharose, and in the grass pollen/*Der p 1* heterologous cross-linking assay (Fig. 5). Binding of *Der p 1* in the antigen-binding test was decreased (≈ 10 -fold) after depletion of IgG4 antibodies; depletion of IgE, or incubation of the serum with control Sepharose (glycine-inactivated Sepharose), did not decrease *Der p 1* binding. By the removal of IgG4, the bispecific binding activity disappeared. This bispecific binding activity remained when IgE was removed, or when the sample was incubated with

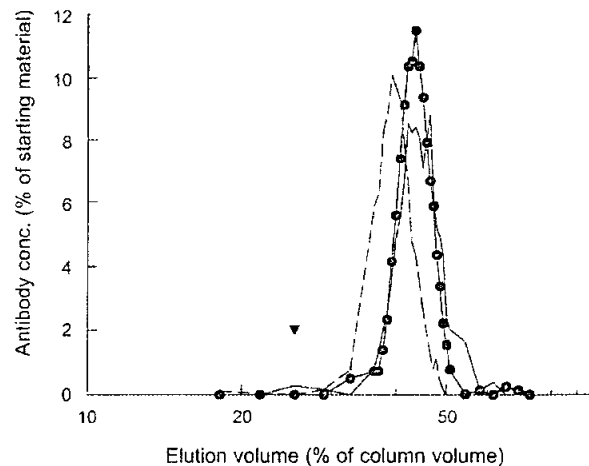


Figure 4. Gel filtration of serum #741. Serum #741, 200 μ l was fractionated on an ACA 34 column. The fractions were tested for total amounts of IgE (180 000 MW, broken line), *Der p 1*-specific IgG (solid line) and for the binding in the heterologous binding assay (●). The void volume is indicated by ▼. The results are expressed as percentage of starting material.

glycine-inactivated Sepharose. These observations demonstrate that IgG4 antibodies cause the bispecific binding of allergen in this serum.

DISCUSSION

Our results indicated the presence of bispecific antibodies in sera from allergic patients receiving immunotherapy (Table 1). We considered four trivial explanations of the apparent bispecificity:

- 1 Contamination of the grass pollen–Sepharose with allergen *Der p 1*;
- 2 Cross-reactivity of a grass pollen allergen with the house dust mite allergen *Der p 1*;

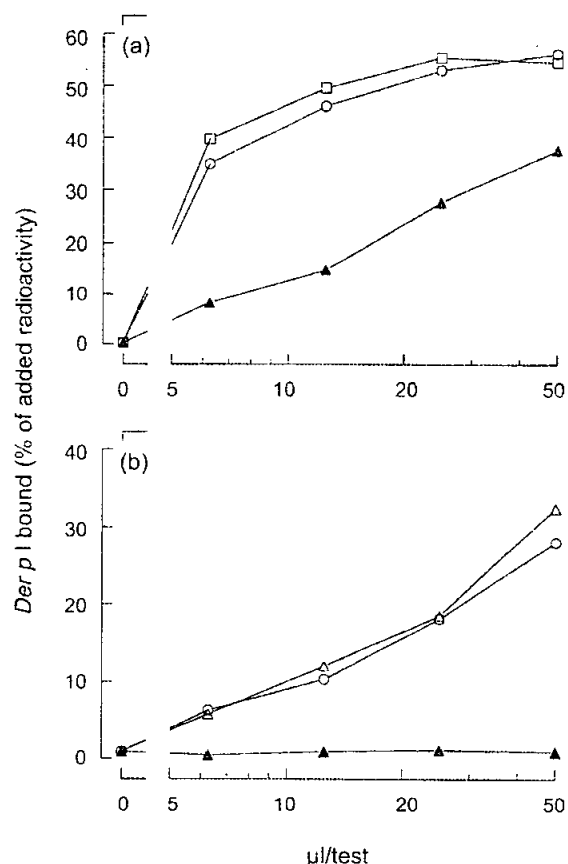


Figure 5. Depletion of IgG4 antibodies from serum #741. Serum #741 was depleted for IgG4 ($\blacktriangle$). As control, a sample was also depleted for IgE ($\square$) and incubated with control Sepharose ($\circ$). The three samples were tested for the amount of *Der p* I-specific IgG (a) and for the binding activity in the heterologous binding assay (b).

3 Antibody aggregation;

4 Non-immune binding of IgG4 to Sepharose-coupled grass pollen.

First, the bispecific binding was not caused by *Der p* I contamination of the grass pollen-Sepharose. This was tested using a hyperimmune rabbit antiserum (Fig. 3).

Second, if the observed bispecificity of IgG4 was due to cross-reactivity, the bispecific sera would show higher binding to Sepharose-coupled mite extract than to Sepharose-coupled grass pollen extract. Even if 100% of the antibodies that reacted with radiolabelled *Der p* I were cross-reactive with grass pollen extract, the cross-reactive binding of these *Der p* I-reactive antibodies could not be higher to grass pollen than to the homologous antigen, in this case: to Sepharose-coupled *Der p* I (or to Sepharose-coupled mite extract). However, we observed that the *Der p* I-binding activity of plasma #741B was approximately threefold lower to optimally coupled mite-extract than to Sepharose-coupled grass pollen (data not shown). This excludes cross-reactivity as explanation for the observed bispecificity. Furthermore, no grass pollen allergen has been described to be cross-reactive with the house dust mite allergen *Der p* I; IgE reactivity is absent in the majority of mite allergic sera. The bispecific binding described in this

paper was not caused by, but could easily be mistaken for, cross-reactivity of allergens. In addition, in sera from patients treated for grass pollen allergy, bispecific antibodies to two non-homologous grass pollen allergens, *Lol p* I and *Lol p* V, were observed (R. van Ree, manuscript in preparation). Bispecific antibodies were also shown in sera from patients receiving immunotherapy for other allergen combinations than grass pollen and house dust mite and were measured to diphtheria and tetanus toxoid (data not shown).

Third, the gel filtration experiment indicated that bispecific antibodies were monomeric IgG molecules, and not antibody aggregates (Fig. 4).

Fourth, the absence of binding of serum #748 (with extremely high levels of *Der p* I-specific IgG4 but with negligible levels of IgG4 to grass pollen) in the heterologous cross-linking assay, excluded non-immune binding of the antibodies to the grass pollen as explanation. The absence of binding of the tested sera to control Sepharose (Table 1) also showed that the binding in the heterologous two-site assay was not caused by a non-specific interaction with Sepharose.

Depletion of the IgG4 antibodies from the serum clearly showed that the bispecific binding was largely, if not completely, caused by IgG4 antibodies (Fig. 5b). A possible explanation of the bispecificity of IgG4 antibodies can be found in some structural features of IgG4. IgG4 half-molecules (containing one heavy and one light chain when analysed on non-reducing sodium dodecyl sulphate-polyacrylamide gel electrophoresis) were noted by several investigators.²⁰⁻²³ These half-molecules are virtually absent in IgG4 with a mutation of a single amino acid (serine 241 to proline, amino acid numbering according to Kabat *et al.*²⁴) in the hinge region in IgG4²⁵ resulting in a closer resemblance to the IgG1 hinge region. This incomplete formation of interheavy chain disulphide bonds might make IgG4 antibodies susceptible to exchange of half-molecules. Exchange in a pool of polyclonal IgG4 would result in bispecific antibodies that behave as monovalent antibodies towards a single antigen. In contrast, monoclonal chimeric IgG4 antibodies would remain bivalent. The bivalency of monoclonal chimeric IgG4 antibodies was found both in electron microscopic analysis²⁶ and in radioimmunoassay (shown in Fig. 2).

The simplest explanation of the bispecificity of IgG4 is the above-mentioned postsecretion exchange of IgG4 half-molecules. An alternative, but less likely explanation is failure of allelic exclusion. This explanation would require an unprecedented phase in the development of IgG4-producing B cells, but would be in line with the observation that *in vitro* incubation of mixtures of chimeric antibodies did not result in bispecific reactions. Also, *in vitro* culturing of a mixture of the nitrophenyl (NP) and *Der p* II-specific IgG4 hybridoma cell lines did not induce bispecific antibodies (not shown).

The postulated physiological mechanism of generation of bivalency *in vivo*, by IgG4 half-molecule exchange, needs further study.

The presence of bispecific IgG4 antibodies creates two new questions:

- 1 What is the mechanism of this half-molecule exchange?
- 2 Is there any (patho-)physiological significance of this bispecificity, for instance in regard to helminth infections and to allergen-specific immunotherapy, or is the generation

of bispecific antibodies a mechanism to generate monovalent antibodies. Monovalent antibodies might be beneficial to inhibit continuous immune-inflammation.

Our data indicate that the apparent monovalency of circulating IgG4 antibodies is owing to bispecificity. This indicates that results obtained with IgG4 antibodies have to be analysed with care. The hypotheses proposed, an intracellular failure of allelic exclusion or an extracellular mechanism involving the exchange of IgG4 half-molecules would explain three observations: first, the apparent monovalency of polyclonal IgG4; second, the difference in valency between polyclonal and monoclonal IgG4, and third, the bispecific activity of IgG4 in selected sera.

ACKNOWLEDGMENTS

The authors like to thank L. A. Aarden and E. J. Nieuwenhuys for stimulating discussions and J. Akkerdaas, A. van Leeuwen, M. G. Valkhof and E. Vermeulen for their help with the serological assays. This work was supported by a research grant from the Netherlands Asthma Foundation grant no. 91-35.

REFERENCES

1. AALBERSE R.C., VAN DER GAAG R. & VAN LEEUWEN J. (1983) Serological aspects of IgG4 antibodies I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* **130**, 722.
2. VAN DER ZEE J.S., VAN SWIETEN P. & AALBERSE R.C. (1986) Serological aspects of IgG4 antibodies. II. IgG4 antibodies form small, nonprecipitating immune complexes due to functional monovalency. *J Immunol* **137**, 3566.
3. BRUEGGEMANN M., WILLIAMS G.T., BINDON C.I. *et al.* (1987) Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. *J Exp Med* **166**, 1351.
4. GREENWOOD J., CLARK M. & WALDMANN H. (1993) Structural motifs involved in human IgG antibody effector functions. *Eur J Immunol* **23**, 1098.
5. ISAACS J.D., CLARK M.R., GREENWOOD J. & WALDMANN H. (1992) Therapy with monoclonal antibodies. An in vivo model for the assessment of therapeutic potential. *J Immunol* **148**, 3062.
6. VAN DER ZEE J.S., VAN SWIETEN P. & AALBERSE R.C. (1986) Inhibition of complement activation by IgG4 antibodies. *Clin Exp Immunol* **64**, 415.
7. LAMBIN P., BOUZOUOMOU A., MURRIETA M. *et al.* (1993) Purification of human IgG4 subclass with allergen-specific blocking activity. *J Immunol Methods* **165**, 99.
8. HAGAN P., BLUMENTHAL U.J., DUNN D., SIMPSON A.J.G. & WILKINS H.A. (1991) Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* **349**, 243.
9. KURNIAWAN A., YAZDANBAKHSH M., VAN REE R. *et al.* (1993) Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis. *J Immunol* **150**, 3941.
10. RIHET P., DEMEURE C.E., DESSEIN A.J. & BOURGOIS A. (1992) Strong serum inhibition of specific IgE correlated to competing IgG4, revealed by a new methodology in subjects from a *S. mansoni* endemic area. *Eur J Immunol* **22**, 2063.
11. HUSSAIN R., POINDEXTER R.W. & OTTESEN E.A. (1992) Control of allergic reactivity in human filariasis. *J Immunol* **148**, 2731.
12. VAN DER GIESSEN M., HOMAN W.L., VAN KERNEBEEK G., AALBERSE R.C. & DIEGES P.H. (1976) Subclass typing of IgG antibodies formed by grass pollen-allergic patients during immunotherapy. *Int Arch Allergy Appl Immunol* **50**, 625.
13. OEHLING A., SANZ M.L. & GARCIA B.E. (1992) Immunological parameters in the immunotherapy follow-up. *Int Arch Allergy Immunol* **99**, 474.
14. FULCHER C.A., DE GRAAF M. & ZIMMERMAN T.S. (1987) FVIII inhibitor IgG subclass and FVIII polypeptide specificity determined by immunoblotting. *Blood* **69**, 1475.
15. SCHUURMAN J., PERDOK G.J., MUELLER G.A. *et al.* (1997) Mouse/human chimeric IgG1 and IgG4 antibodies directed to the house dust mite allergen Der p 2: Use in quantification of allergen specific IgG. *Clin Exp Allergy* **27**, 1095.
16. OVSYANNIKOVA I.G., VAILES L.D., LI Y., HEYMANN P.W. & CHAPMAN M.D. (1994) Monoclonal antibodies to group II Dermatophagoides spp. allergens: murine immune response, epitope analysis, and development of a two-site ELISA. *J Allergy Clin Immunol* **94**, 537.
17. VAN DER ZEE J.S., VAN SWIETEN P., JANSEN H.M. & AALBERSE R.C. (1988) Skin tests and histamine release with P1-depleted D. pteronyssinus body extracts and purified P1. *J Allergy Clin Immunol* **81**, 884.
18. VAN REE R., CLEMENS J.C.J., AALBERS M., STAPEL S.O. & AALBERSE R.C. (1989) Characterization with monoclonal and polyclonal antibodies of a new major allergen from grass pollen in the group I molecular weight range. *J Allergy Clin Immunol* **83**, 144.
19. MUELLER G.A., SMITH A.M., WILLIAMS D.C. *et al.* (1997) Expression and secondary structure determination by NMR methods of the major house dust mite allergen Der p 2. *J Biol Chem* **272**, 26893.
20. PETERSEN J.G.L. & DORRINGTON K.J. (1974) An in vitro system for studying the kinetics of interchain disulphide bond formation in immunoglobulin G. *J Biol Chem* **249**, 5633.
21. KING D.J., ADAIR J.R., ANGAL S. *et al.* (1992) Expression, purification and characterization of a mouse-human chimeric antibody and chimeric Fab fragment. *Biochem J* **281**, 317.
22. COLCHER D., MILENIC D., ROSELLI M. *et al.* (1994) Characterization and biodistribution of recombinant/chimeric constructs of monoclonal antibody B72.3. *Cancer Res* **49**, 1738.
23. TURNER M.W., BENNICHI H.H. & NATVIG J.B. (1970) Pepsin digestion of human G-myeloma proteins of different subclasses II. Immunochemical investigations of the products of pepsin digestion. *Clin Exp Immunol* **7**, 627.
24. KABAT E.A., WU T.T., REID-MILLER M., PERRY H.M. & GOTTESMAN K.S. (1987) Sequences of proteins of immunological interest, edn 4. US Department of Health and Human Services, Public Health Service, National Institutes of Health.
25. ANGAL S., KING D.J., BODMER M.W. *et al.* (1993) A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human antibody. *Mol Immunol* **30**, 105.
26. PHILLIPS M.L., TAO M.H., MORRISON S.L. & SCHUMAKER V.N. (1994) Human/mouse chimeric monoclonal antibodies with human IgG1, IgG2, IgG3 and IgG4 constant domains: electron microscopic and hydrodynamic characterization. *Mol Immunol* **31**, 1201.
27. AALBERSE R.C., DIEGES P.H., KNUL-BRETLOVA V., VOOREN P., AALBERS M. & VAN LEEUWEN J. (1983) IgG4 as blocking antibody. *Clin Rev Allerg* **1**, 289.

2020
Bogotá, D.C.,

Doctor:
Emilio Ferrero
Apoderado

Oficio N°: 09218

ASUNTO:	Radicación PCT N°	08-105676
	Trámite	011
	Evento	378
	Actuación	440
	Folios	010

Con fundamento en lo previsto por el artículo 43 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica que la oposición presentada por la doctora Cielo Ángela Peña Rodríguez, en su calidad de apoderada de Procaps S.A., reúne los requisitos formales exigidos por la ley al momento de su presentación.

En cumplimiento de lo dispuesto en la citada norma, se le concede el término de sesenta (60) días hábiles siguientes a la fecha de notificación del presente oficio, para que haga valer sus argumentaciones y presente pruebas.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


ALIX CARMENZA CESPEDES DE VERGEL
Directora de Nuevas Creaciones (C).

El anterior oficio fue notificado por fijación en lista de fecha, 23 JUL. 2010.

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