Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 10-023468- -00003-0000

Fecha: 2011-06-16 16:02:41 Dep: 2020 DIR.NUEVASCR
Tra: 11 PATENTE/MI Eve: 378 FASENACIONALI
Act: 400 OPOSICIOBSERVTEP Folios: 48

FORMULARIO ÚNICO DE OPOSICIÓN

① OPOSICIÓN A :

- [X] Patente de Invención.
- [ ] Patente de Modelo de Utilidad.
- [ ] Diseño Industrial.
- [ ] Esquema de Trazado para Circuitos Integrados.
- [ ] Marcas de Productos o Servicios.
- [ ] Marca Colectiva.
- [ ] Marca de Certificación.
- [ ] Lema Comercial.

② SOLICITUD PUBLICADA

Número del expediente: 10 – 023.468
Solicitante: REGENERON PHARMACEUTICALS, INC
Apoderado: LUZ CLEMENCIA SUAREZ DE PAEZ
Título de la nueva creación o denominación del signo: ANTICUERPOS HUMANOS CONTRA CD20 HUMANO Y MÉTODO PARA UTILIZARLOS"
Gaceta: 626

③ OPOSICIÓN PRESENTADA POR:

SOLICITANTE	Nombre: **PROCAPS S.A.** Dirección: **CALLE 80 No. 78b-201** Nacionalidad o Domicilio: **COLOMBIA** Lugar de Constitución: **BARRANQUILLA** Teléfono: **371 99 00** Fax: **373 0815** E-mail:	**IDENTIFICACIÓN** C.C. [ ] NIT [X] C.E. [ ] Otro [ ] Cual ____ Número **890.106.527-5**
REPRESENTANTE O APODERADO	Nombre: **ELIANA ISAURA MORENO BOHORQUEZ** Dirección: **CARRERA 104 N°119-95 OF 104** Teléfono: **2131213** Fax: **6121979** E-mail: **negocios@optimum-co.com**	**IDENTIFICACIÓN** C.C. [X] NIT [ ] C.E. [ ] Otro [ ] Cual ____ Número **51.975.664** TP **83823**

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**

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

⑦

NOMBRE: Edwin E. Moreno B.

FIRMA:

C.C. 51.975.664 **T.P.** 83823

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

Optimum

Derecho de la Competencia, Propiedad Industrial y Comercio Exterior

Señor Jefe,

DIRECCION DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

Referencia	: Expediente N° 10 23468
Asunto	: Oposición a la solicitud de patente de Invención titulada "ANTICUERPOS HUMANOS CONTRA CD20 HUMANO Y METODO PARA UTILIZARLOS"
Solicitante	: REGENERON PHARMACEUTICALS INC.
Opositora	: PROCAPS S.A.

Publicada en la Gaceta de la Propiedad Industrial

N° 626 del 22 de marzo de 2011

Yo, *ELIANA ISAURA MORENO BOHORQUEZ,* abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. *83823* 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 "ANTICUERPOS HUMANOS CONTRA CD20 HUMANO Y METODO PARA UTILIZARLOS", cuyos titulares son **REGENERON 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 ANTICUERPOS HUMANOS AQUITE CD-20. 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 26 de febrero de 2010 reivindicando prioridad bajo la solicitud US20070962811 del 2007-07-31; y fue publicada en Colombia, en la Gaceta de la Propiedad Industrial N° 626 del 22 de marzo de 2011, cuya publicación internacional corresponde a la WO2009018411 del 2009-02-05.

2.2. La solicitud colombiana 10 23468 objeto de la presente oposición se refiere a un anticuerpo humano o un fragmento de unión al antígeno de un anticuerpo que se une específicamente al CD20 humano y que es capaz de inducir una citotoxicidad dependiente del complemento (CDC), y que es capaz de aumentar el tiempo de

supervivencia sin síntomas entre aproximadamente 2 veces a aproximadamente 9 veces más o mayor, con relación a animales control tratados en un modelo de ratón del linfoma humano. El anticuerpo o su fragmento de unión al antígeno es útil para un método terapéutico para tratar una enfermedad o un trastorno mediado por CD20, como por ejemplo el linfoma no hodgkiniano, la artritis reumatoide, el lupus eritematoso sistémico, la enfermedad de Crohn, la leucemia linfocítica crónica, y las enfermedades inflamatorias.

2.3. En primera instancia, se debe advertir que en el capítulo reivindicatorio de la solicitud objeto de la presente oposición, se hace referencia a los usos de la composición, no sólo a la composición como tal 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 al uso no sería patentable.

2.4. 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: **US 2004167319** publicada el 26 de agosto de 2004.

2.5. Ahora bien, el documento D1, describe de forma particular en los ejemplos 6 a 11; y Figuras 10 a 19, 38 a 40 y 53, la producción y caracterización de anticuerpos humanos anti CD20 2F2 (como ofatumumab) y demuestra que dicho anticuerpo ha incrementado la actividad CDC y ha resultado en aumento de la supervivencia de los ratones con ID combinada grave los linfomas de células B Daudi o Tanoue en comparación con rituximab.

2.6. Tomando el documento D1 como muestra del estado del arte anterior a la solicitud colombiana 10 23468, no es posible reconocer altura inventiva, ya que, teniendo en cuenta que los anticuerpos de la solicitud objeto de la presente oposición difieren del anticuerpo 2F2 presentado en el documento D1 únicamente en su CDP. y en secuencias de la región variable, donde la mejora en tiempo de supervivencia presente en el anticuerpo 10F2-13 de la solicitud colombiana 10 23468 con respecto a al 2F2 del documento D1 es inesperada, sería obvio para un experto en la materia a partir de dicho documento D1 por sí solo, concebir un anticuerpo humano o un fragmento de unión al antígeno de un anticuerpo que se une específicamente al CD20 humano y que es capaz de inducir una citotoxicidad dependiente del complemento (CDC) y su utilidad para un método terapéutico para tratar una enfermedad o un trastorno mediado por CD20, como por ejemplo el linfoma no hodgkiniano, la artritis reumatoide, el lupus eritematoso sistémico, la enfermedad de Crohn, la leucemia linfocítica crónica, y las enfermedades inflamatorias.

2.7. En consecuencia y de acuerdo a lo descrito anteriormente, se deriva que la solicitud colombiana 10 23468 no puede pretender un mono polio por patente toda vez que lo que pretende no cumple con dos de los tres requisitos necesarios para su protección.

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

2.9. <u>CARENCIA DE ALTURA INVENTIVA</u>. 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, 31 de julio de 2007, a partir del documento D1 por si solo, un experto en la materia puede llegar de manera evidente a concebir un anticuerpo humano o un fragmento de unión al antígeno de un anticuerpo que se une específicamente al CD20 humano que difiere del 2F2 del documento D1 solo en su CDR y en secuencias de la región variable, que es capaz de inducir una citotoxicidad dependiente del complemento (CDC) y su utilidad para un método terapéutico para tratar una enfermedad o un trastorno mediado por CD20, como por ejemplo el linfoma no hodgkiniano, la artritis reumatoide, el lupus eritematoso sistémico, la enfermedad de Crohn, la leucemia linfocítica crónica, y las enfermedades inflamatorias; sobre lo cual busca protección en el capítulo reivindicatorio.

2.9.1. <u>CARENCIA DE APLICACIÓN INDUSTRIAL</u> 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 reclaman usos y estos no son aplicables industrialmente porque primero no se obtiene un producto o proceso; y segundo no es reproducible o repetible a escala industrial. Por lo tanto, el objeto de la presente invención referido a los usos, no cumple con el requisito de la aplicación industrial.

2.10. 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.

2.11. **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 "ANTICUERPOS HUMANOS CONTRA CD20 HUMANO Y METODO PARA UTILIZARLOS", cuyos titulares son REGENERON PHARMACEUTICALS INC, solicitud Colombiana que apareció publicada en la Gaceta de la Propiedad Industrial N° 626 del 22 de marzo de 2011.**

3. PRUEBAS

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

- D1: **US 2004167319** publicada el 26 de agosto de 2004.

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: **US 2004167319** publicada el 26 de agosto de 2004.

Optimum

Derecho de la Competencia, Propiedad Industrial y Comercio Exterior

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 Nº 119-95, Of. 104 de esta ciudad de Bogotá.

Señor Superintendente,

ELIANA ISAURA MORENO BOHORQUEZ
C.C. 51'975.664 de Bogotá
T.P.A. 83823 del C.S. de la J.

Carrera 13 Nº 119-95, Of. 104 Tel. (571) 2 13 12 13 - 6 20 50 21 - Fax. (571) 6 12 19 79
E-mail: negocios@optimum-co.com - www.optimum-co.com
Bogotá Colombia

PROCAPS

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 **ELIANA ISAURA MORENO BOHORQUEZ**, abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. 83823 **del C.S. de la J.**, poder especial, amplio y suficiente, para formular oposiciones u observaciones a la solicitud de patente No. **10 023.468,** titulada **"ANTICUERPOS HUMANOS CONTRA CD20 HUMANO Y MÉTODO PARA UTILIZARLOS",** cuyo titular es **REGENERON PHARMACEUTICALS, INC,** publicada en la gaceta No. 626 del 22 de marzo de 2011.

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 administrativamente 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 8 días del mes de Junio de 2011.

PROCAPS S.A.

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

APROBADO
7 - Jun. 2011
PROCAPS S.A

Acepto:

ELIANA ISAURA MORENO BOHORQUEZ
C.C. 51'975.664 de Bogotá
T.P.A. 83823 del C.S. de la J.

NOTARIA 6

NOTARIA TERCERA DEL CIRCULO DE BARRANQUILLA

DILIGENCIA DE RECONOCIMIENTO

Ante el Notario Tercero de Barranquilla se presento:

Walter Tarazona Valenzuela

identificado con: C: 16 255923 Palmira

Y declaro que el contenido del anterior Documento es cierto y suya la firma que lo refrenda.

09 JUN 2011

Barranquilla:

NOTARIA TERCERA DEL CIRCULO DE BARRANQUILLA
ALFONSO LUIS AVILA FADUL
NOTARIO

12

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 11 - 0058325

Bogotá D.C., Junio 16 de 2011 - 15:42:55

RECIBIDO DE : PROCAPS S.A. NI 890.106.527

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	382186162	16/06/2011	304.000.00

*** Conceptos Pagados ***

CANT.	PENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-04 PRESENTACION DE OBSERVACIONES A SOLICITUDES	12 MARCAS Y LEMAS COMERCIALES	304.000.00	304.000.00
				$304.000.00

==========

SON: **TRESCIENTOS CUATRO MIL PESOS MONEDA CORRIENTE***

Responsable: _____

-***-

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 10-023468- -00003-0000
Fecha: 2011-06-16 16:02:41 Dep. 2020 DIR.NUEVASOR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 400 OPOSIOBSERVTER Folios: 48

03-05013037 024803 PROCAPS S.A.
Barranquilla, 01 de Junio de 2011 Hr:11:33:49

No. 16014663

********** 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
03**********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
PROCAPS S.A.----------
NIT: 890.106.527-5.

==========
RENUEVE SU MATRICULA MERCANTIL ANTES DEL 31 DE MARZO DE 2011. SI NO ESTA AL DIA EN AÑOS ANTERIORES, ACOJASE A LOS BENEFICIOS TEMPORALES DE LA LEY DE FORMALIZACION. ¡PREGUNTE POR ELLOS!.
MAS INFORMACIÓN EN NUESTRA LINEA DE ATENCION AL CLIENTE: 3617555
==========

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, fué 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

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

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

PROCAPS S.A.

NIT: 890.106.527-5.

"PRODUCTORA DE CAPSULAS DE GELATINA S.A. "PROCAPS S.A.".----------

C E R T I F I C A

Que por Escritura Pública No. 3,615 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

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

03-05013027 024802 PROCAPS S.A.
Barranquilla, 01 de Junio de 2011 Hr:11:32:49

No. 16014664

CAMARA DE COMERCIO DE BARRANQUILLA

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
03**********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
PROCAPS S.A.----------
NIT: 890.106.527-5.

se rige por las siguientes disposiciones:
DENOMINACION O RAZON SOCIAL:
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.
Correo electrónico: mlozada@procaps.com.co
Pagina Web:
Telefono: 3719272

C E R T I F I C A

DUFACION: 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 fabicacion, importacion y comercialicacion 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, alimen ticias y cosmeticas, asi como la comercialicacion 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,

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

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, 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 gravamenes 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 gravamenes reales sobre sus bienes de cualquier naturaleza.----------

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		
$**********1,298,000,000	**********1,298,000	**********1,000
Pagado		
$**********1,298,000,000	**********1,298,000	**********1,000

C E R T I F I C A

ADMINISTRACION: La direccion y administracion de la sociedad corresponderá 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

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

No. 16014665

CAMARA DE COMERCIO DE BARRANQUILLA

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
03**********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
PROCAPS S.A.----------
NIT: 890.106.527-5.

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.
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 siguentes 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 gravamenes sobre cualquiera de los anteriores, facultades que radican exclusiva y privativamente en cabeza del Presidente. Las demas funciones que le corresponda como Representante legal por disposicion de la ley, de estos estatutos o de la Junta Directiva. El Vicepresidente tendra (1) suplente, designado por la Junra 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 gravamenes 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 CC.*******7,463,982

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

03-05013027 024802 PROCAPS S.A.
Barranquilla, 01 de Junio de 2011 Hr:11:32:49

No. 16014666

CAMARA DE COMERCIO DE BARRANQUILLA

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
03**********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
PROCAPS S.A.----------
NIT: 890.106.527-5.

2. Minski Gontovnik Meyer CC.*******7,461,324
3. Minski Gontovnik Jose CC.*******8,671,834

Suplentes
1. Minski Sirberblum Elliot CC.******73,219,541
2. Minski Deitel Daniel .**********
3. Minski Sharon PA.*990,313,082,305

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

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.	

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

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

PROCAPS S.A.

NIT: 890.106.527-5.

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 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 Legaql de la sociedad PROCAPS S.A., el presidente ---- debidamente facultado por los Estatutos Sociales y por el Maximo Organo 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, ------

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

CAMARA DE COMERCIO DE BARRANQUILLA

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
03**********

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
PROCAPS S.A.----------
NIT: 890.106.527-5.

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 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 mercancias 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 dia que se realice la respectiva transaccion. Grupo A. WALTER HANAKA TATEHAWA CC.No.16.251.923, CLAUDIA TANGARIFE CASTILLO ---------- CC.No.41.660.293, CARMEN ALICIA FLORES CC.No.22.434.044, IVAN ------ VILLAREAL CAMACHO CC.No.91.301.749, CARMEN ALICIA HERRERA ---------- DIASGRANADOS 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 senor WILLIAM ALBERTO BARROS CERRA ---------- CC.No.8.715.941, para que en nombre y representacion 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 demas documentos relacionados con ellas, ---------- notificarse, aportar y solicitar las pruebas que sean necesarias. c.-Otorgar poder especial amplio y suficiente al senor MARLON ----- TATIS PAREDES CC.No.73.176.874 y al senor WILLIAM ALBERTO BARROS CERRA CC.No.8.715.941, para que en nombre y representacion 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 DIASGRANADOS, CC.No.22.377.540 y al doctor WALTER TANAKA TATEHAWA, CC.No.16.251.923, para que en nombre y ---------- representacion 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: El 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.----------

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. 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 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 carácter 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 por Documento Privado del 10 de Junio de 2010, otorgado en Barranquilla inscrito en esta Cámara de Comercio, el 13 de Julio de 2010 bajo el Nro 4,228 del libro respectivo,----------
consta que el señor RUBEN MISNKI GONTOVNIK, cc. No. 7.463.982 en su condicion de Presidente y representante legal de PROCAPS S.A., ----- confiere poder especial, amplio y suficiente al señor RICARDO DORADO HURTADO cc. No. 79.715.772 para que represente a la sociedad, ante el INVIMA, Fondo Nnacional de estupefacientes e ICA, con el fin que gestione, tramite o presente las diferentes solicitudes y ---------- peticiones de la empresa, segun la competencia de estas entidades.
Por lo anterior, el Señor RICARDO DORADO HURTADO, queda facultado para presentar, gestionar, tramitar y firmar las diferentes -------- solicitudes y peticiones de la empresa, segun la competencia de ---- estas entidades, como tambien presentar y firmar todo los ---------- documentos relacionados con estas solicitudes, notificarse de ------ cualquier acto administrativo que expidan, aportar y solicitar las pruebas que sean necesarias, desistir, y en general a realizar todo

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

03-05013027 024803 PROCAPS S.A.
Barranquilla, 01 de Junio de 2011 Hr:11:32:49

No. 16014668

CAMARA DE COMERCIO DE BARRANQUILLA

C A M A R A D E C O M E R C I O
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CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.
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tramite o acto necesario de tal forma que la sociedad en ningun ---- momento quede sin representacion legal ante estas entidades, en ---- cumplimiento fiel al mandato otorgado.----------

C E R T I F I C A

Que por Documento Privado del 03 de Agosto de 2010, otorgado en Barranquilla inscrito en esta Cámara de Comercio, el 24 de Agosto de 2010 bajo el Nro 4,258 del libro respectivo,---------- consta que RUBEN MINSKI GONTOVNIK, varón, mayor de edad, de ésta vecindad, identificado con la cédula de ciudadanía No.7.463.932, expedida en Barranquilla, en mi condición de Representante legal y/o Presidente de la sociedad PROCAPS S. A., conforme a las -------- facultades otorgadas por los Estatutos Sociales, todo lo cual ------ demuestro con el certificado de existencia y representación legal expedido por la Cámara de Comercio de Barranquilla, que adjunto a este escrito, me permito manifestarles que confiero poder especial, amplio y suficiente al señor MARIO LÓPEZ LEON, varón, mayor de ----- edad, de esta vecindad, identificado con la cédula de ciudadanía No.72.311.558, expedida en Puerto Colombia (Atlántico), para que en nombre y representación de la sociedad, realice los siguientes ----- actos: 1. Endosar las Facturas Nacionales e Internacionales de esas compañías hasta por la suma de UN MIL MILLONES DE PESOS ---------- ($1.000.000.000.00) Moneda Legal. 2. Suscribir Declaraciones ------- Cambiarias, Aduaneras y Tributarias a las que esta obligada la ----- sociedad en el giro ordinario de sus negocios, quedando facultado para notificarse, aportar y solicitar pruebas relacionados con las declaraciones Cambiarias. El señor MARIO LÓPEZ LEÓN, queda --------- facultado para notificarse, aportar y solicitar pruebas, presentar escritos en caso que resulte necesario en general a realizar todo acto, diligencia o tramite que considere indispensable 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: 31 de Marzo de 2011.

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

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

PROCAPS S.A.

NIT: 890.106.527-5.

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.

CAMARA DE COMERCIO DE BARRANQUILLA

US 20040167319A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0167319 A1**

Teeling et al. (43) **Pub. Date: Aug. 26, 2004**

(54) **HUMAN MONOCLONAL ANTIBODIES AGAINST CD20**

(76) Inventors: **Jessica Teeling**, Southampton (GB); **Sigrid Ruuls**, De Bilt (NL); **Martin Glennie**, Southampton (GB); **Jan G.J. van de Winkel**, Zeist (NL); **Paul Parren**, Odyk (NL); **Jorgen Petersen**, Rungsted Kyst (DK); **Ole Baadsgaard**, Malmo (SE); **Haichun Huang**, Fremont, CA (US)

Correspondence Address:
LAHIVE & COCKFIELD, LLP.
28 STATE STREET
BOSTON, MA 02109 (US)

(21) Appl. No.: **10/687,799**

(22) Filed: **Oct. 17, 2003**

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Publication Classification

(51) **Int. Cl.**[7] **C07K 16/28**

(52) **U.S. Cl.** **530/388.22**

(57) **ABSTRACT**

Isolated human monoclonal antibodies which bind to and inhibit human CD20, and related antibody-based compositions and molecules, are disclosed. The human antibodies can be produced by a transfectoma or in a non-human transgenic animal, e.g., a transgenic mouse, capable of producing multiple isotypes of human monoclonal antibodies by undergoing V-D-J recombination and isotype switching. Also disclosed are pharmaceutical compositions comprising the human antibodies, non-human transgenic animals and hybridomas which produce the human antibodies, and therapeutic and diagnostic methods for using the human antibodies.

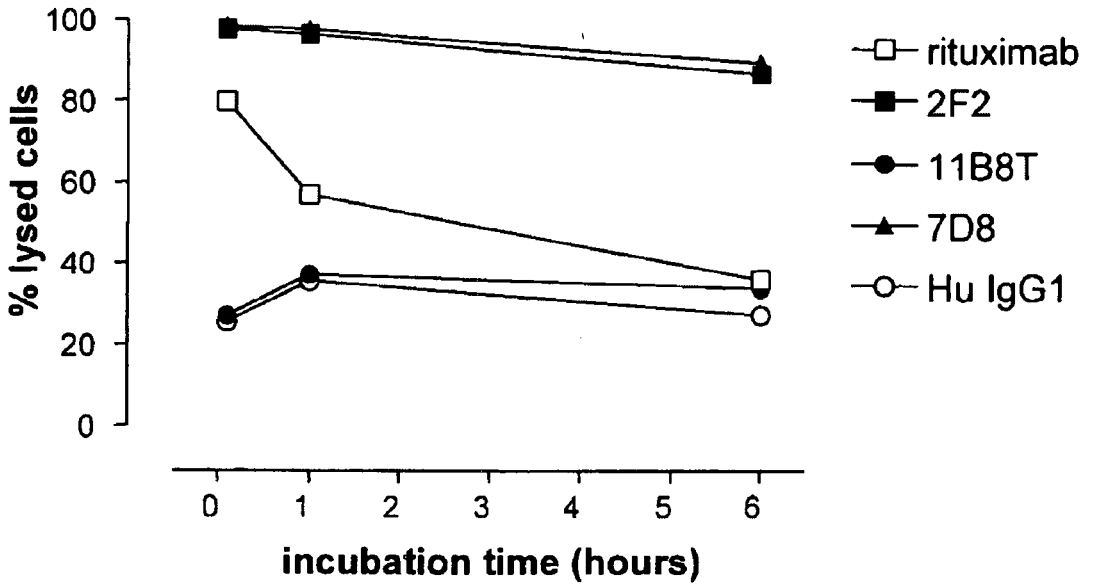


Fig. 10A

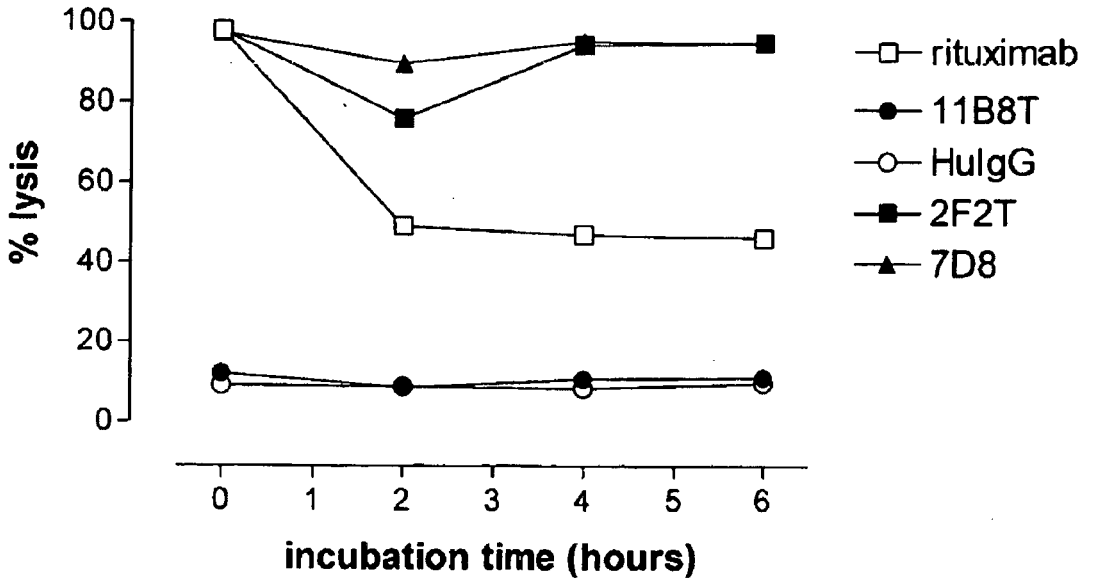


Fig. 10B

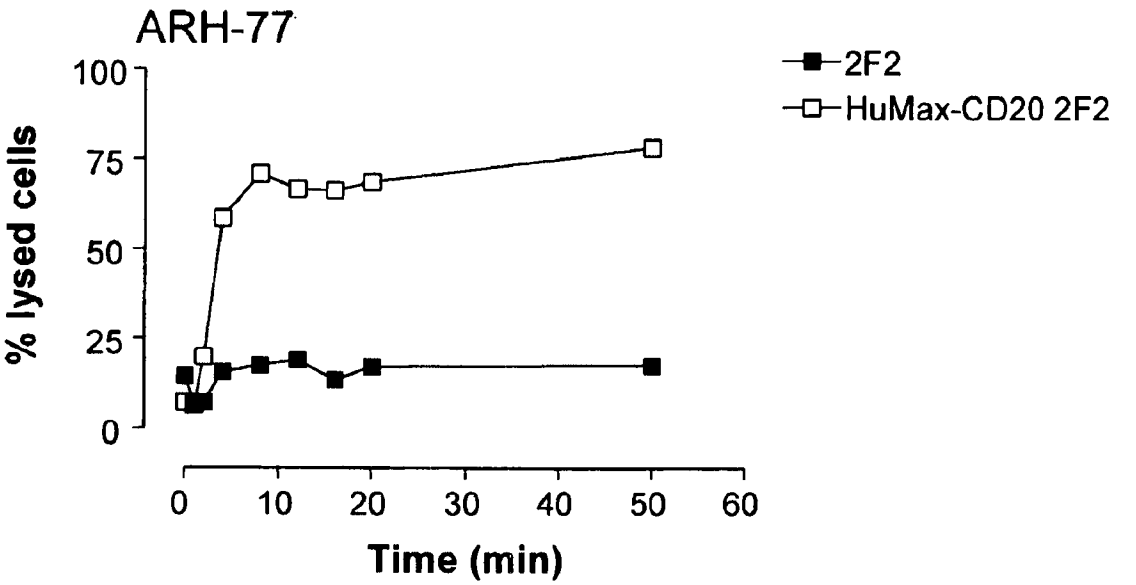


Fig. 11A

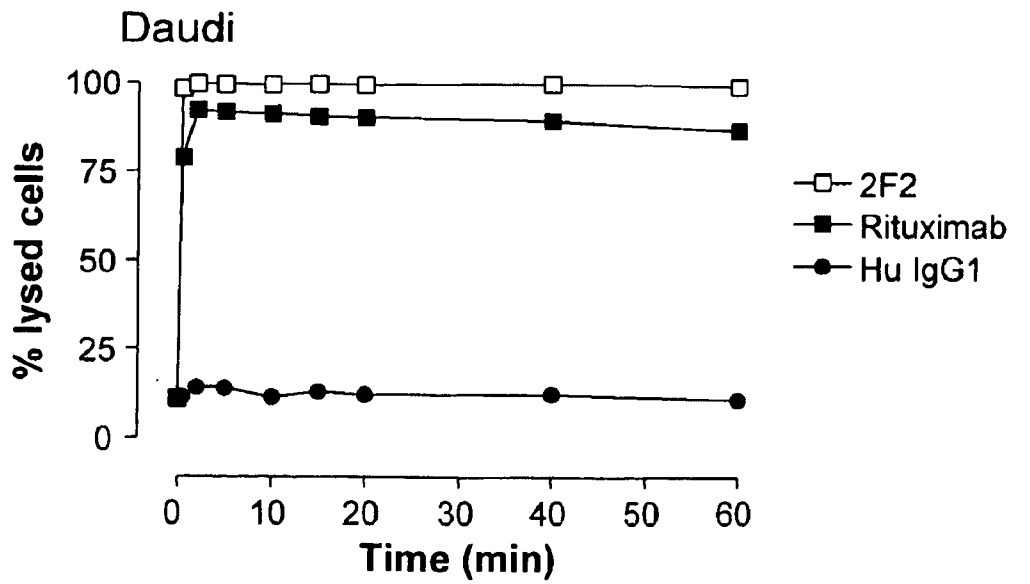


Fig. 11A

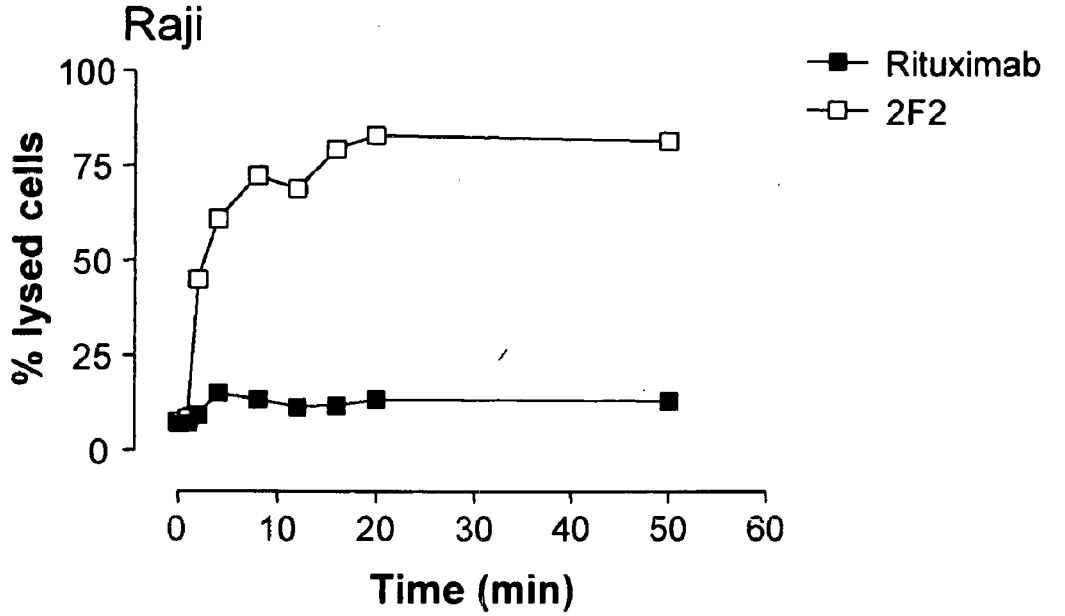


Fig. 11C

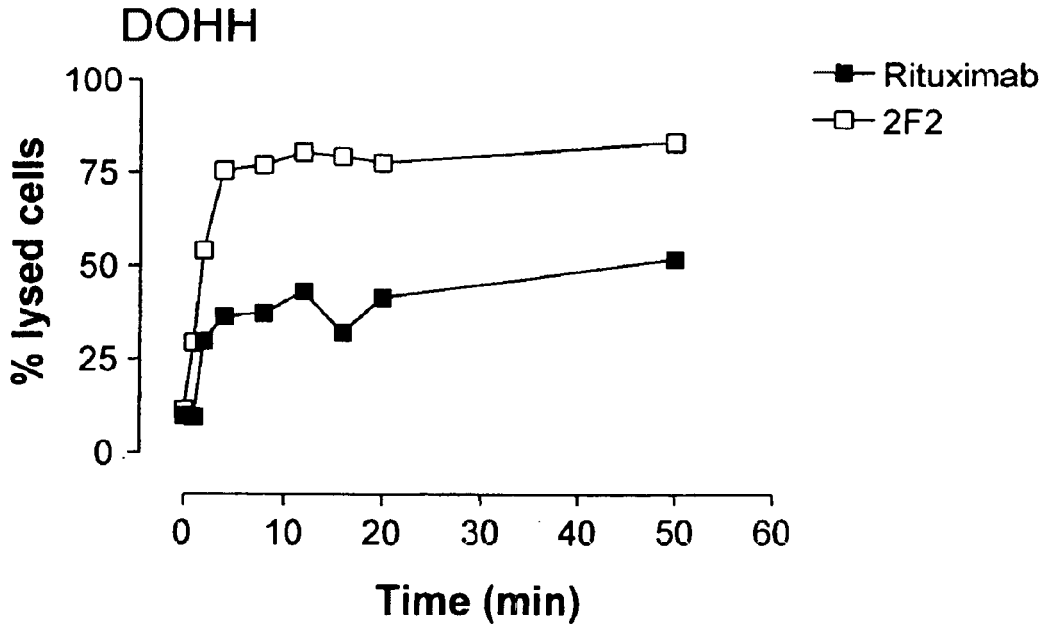


Fig. 11D

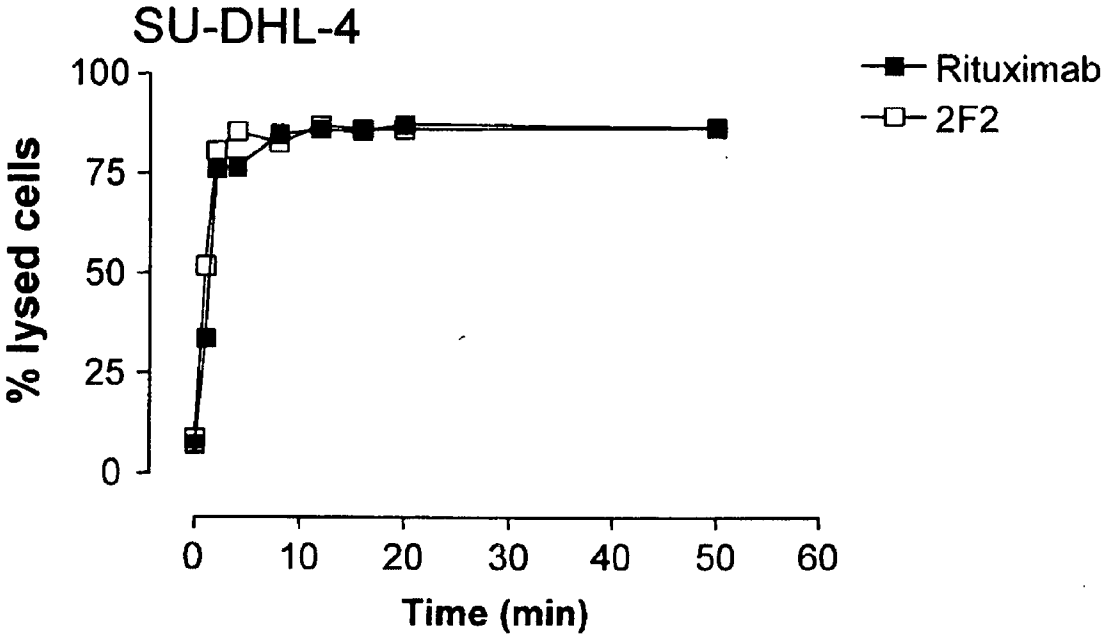

SU-DHL-4
Rituximab
2F2
% lysed cells
100
75
50
25
0
0
10
20
30
40
50
60
Time (min)

Fig. 11E

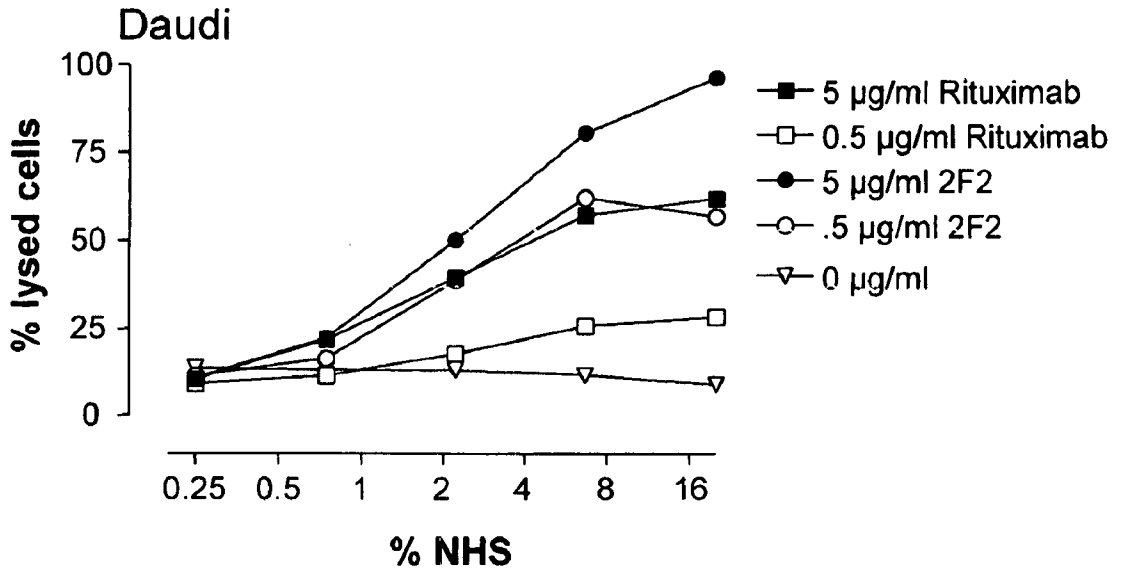


Fig. 12A

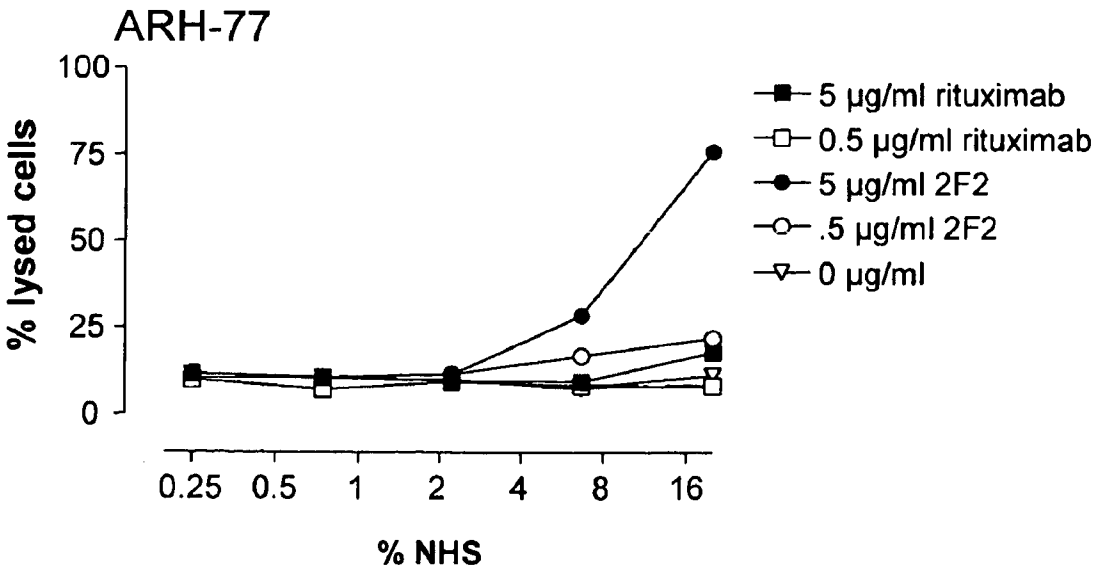


Fig. 12B

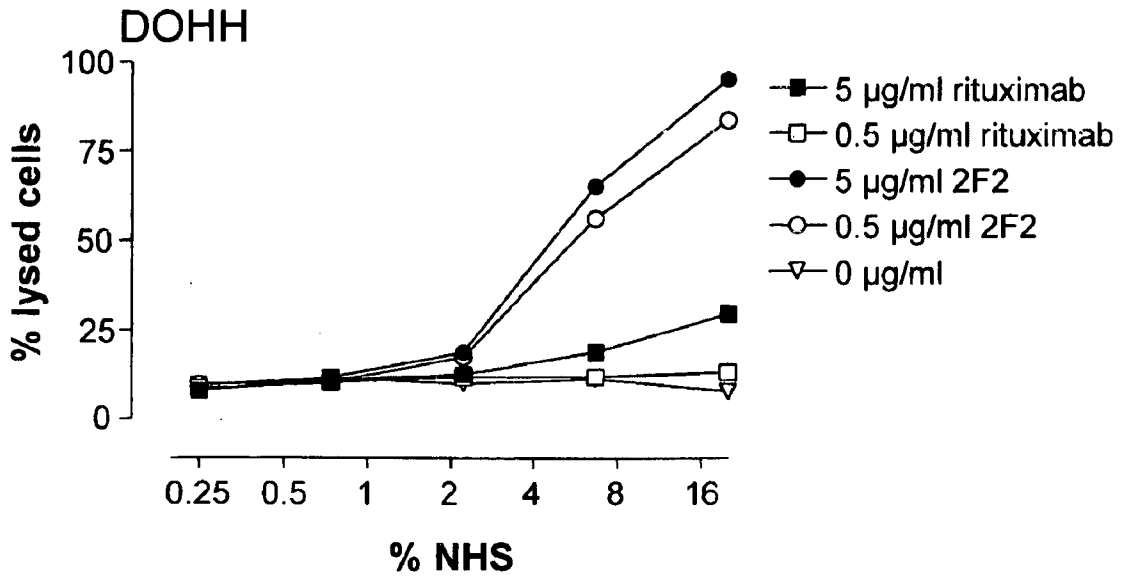


Fig. 12C

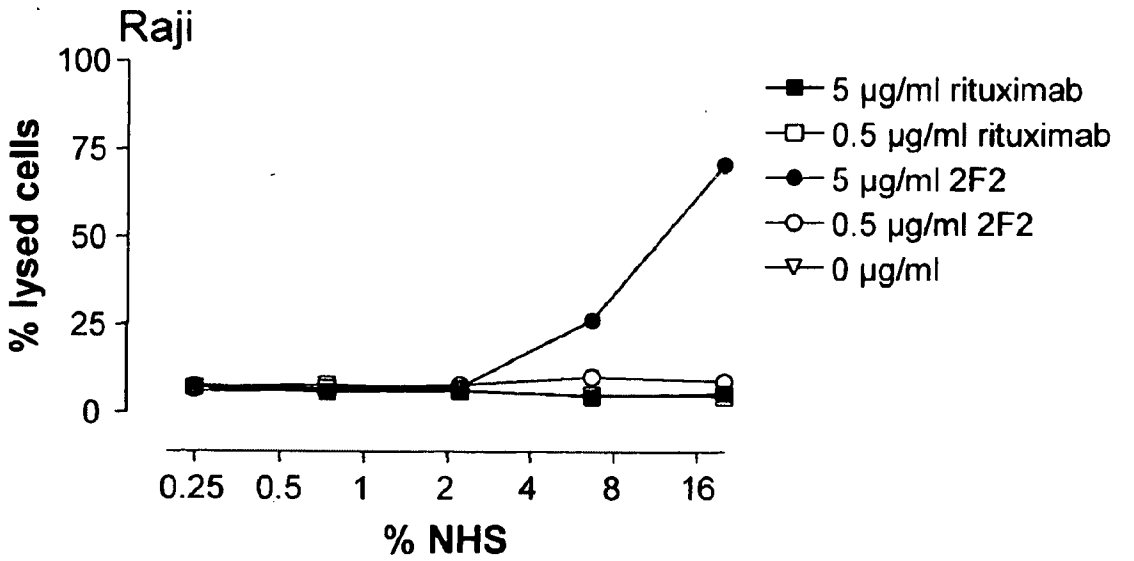


Fig. 12D

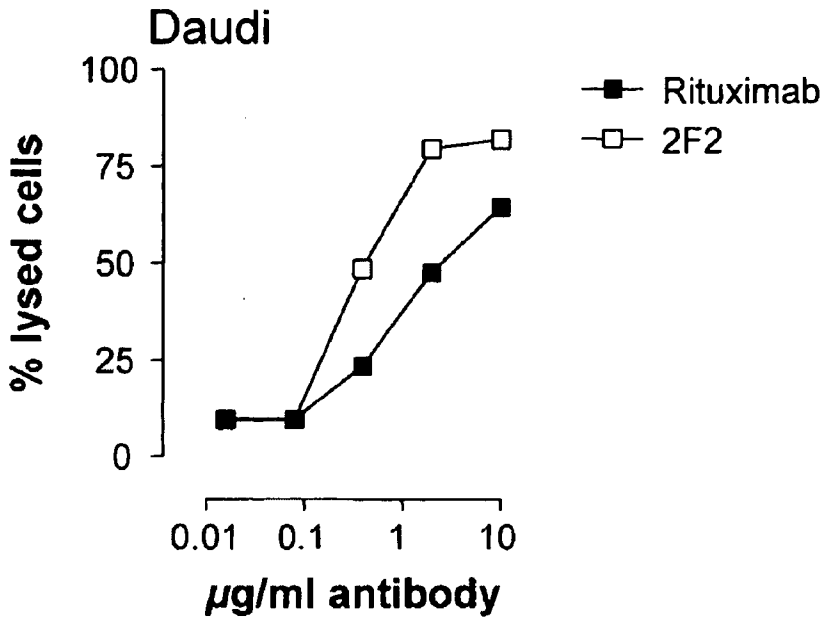


Fig. 13A

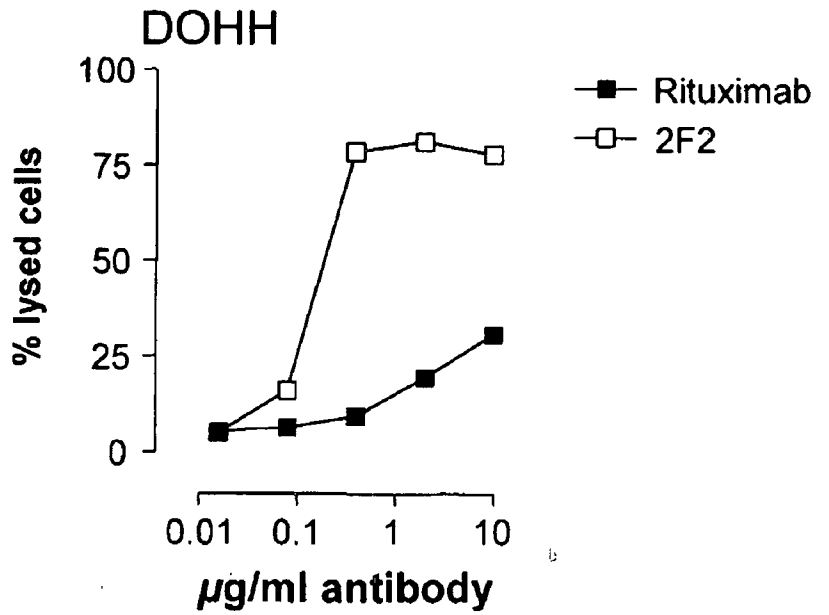


Fig. 13B

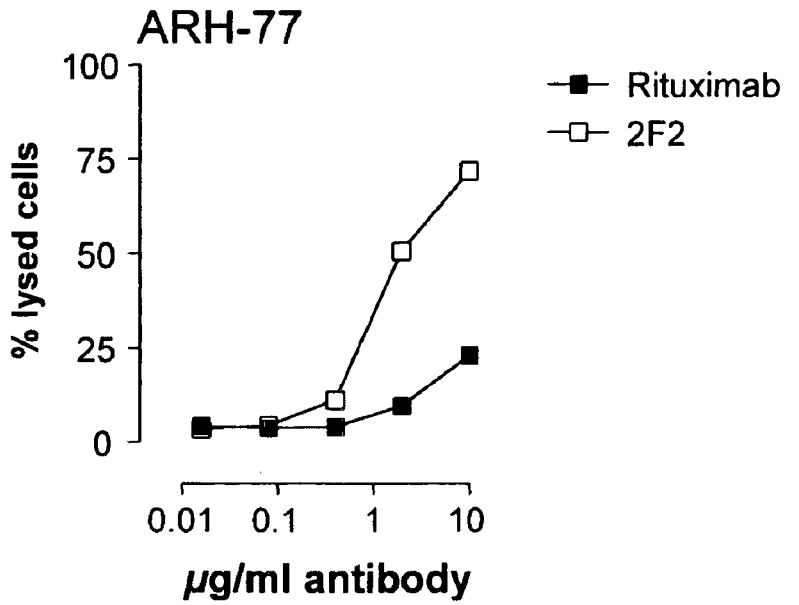


Fig. 13C

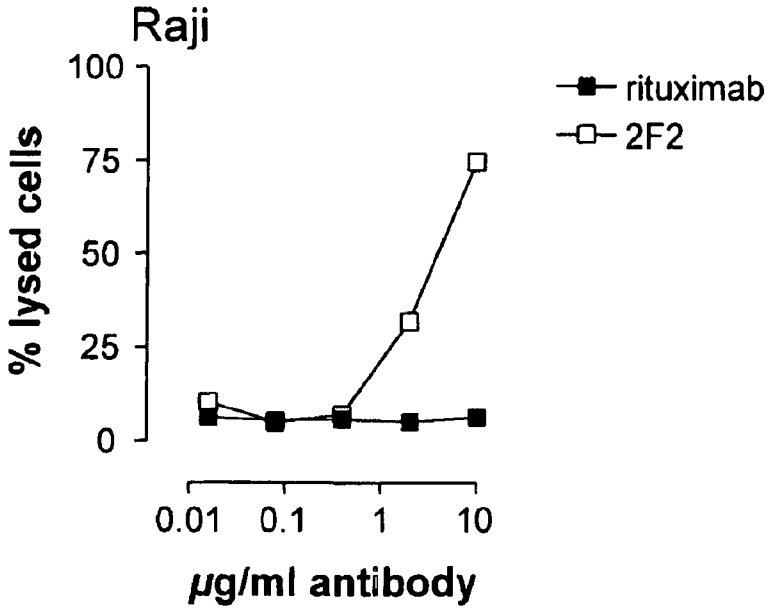


Fig. 13D

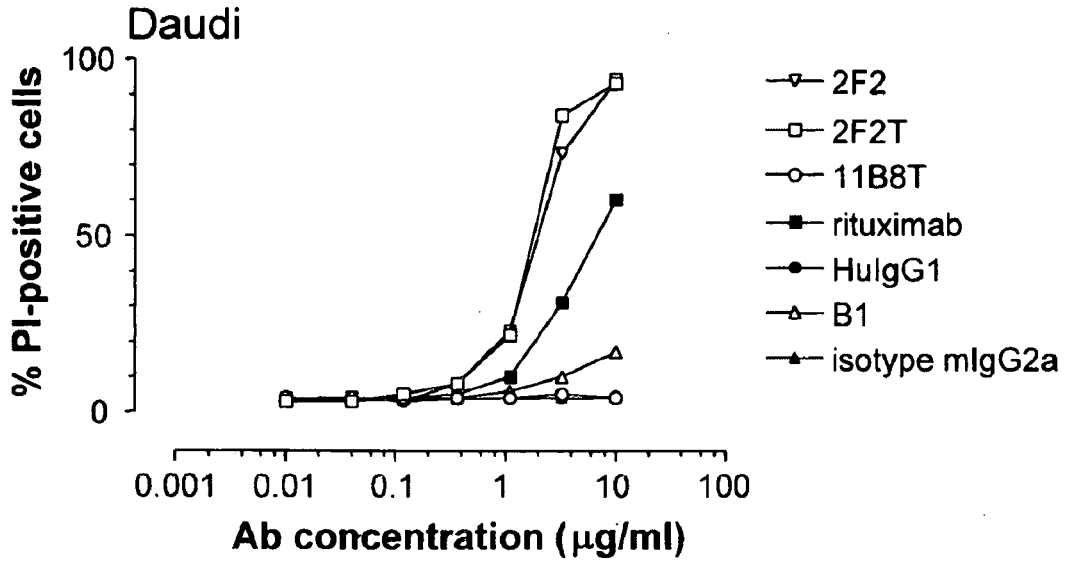


Fig. 14A

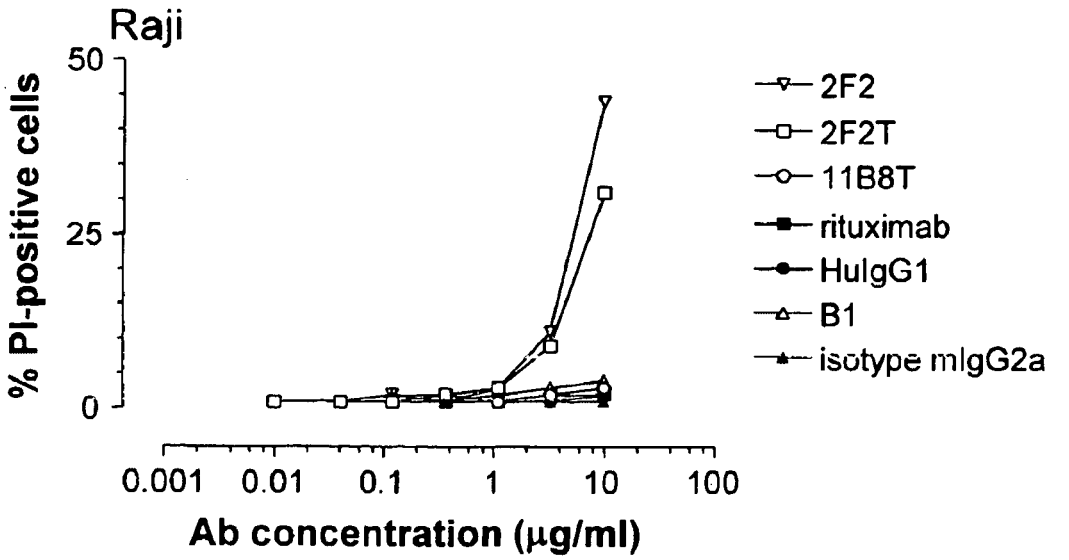
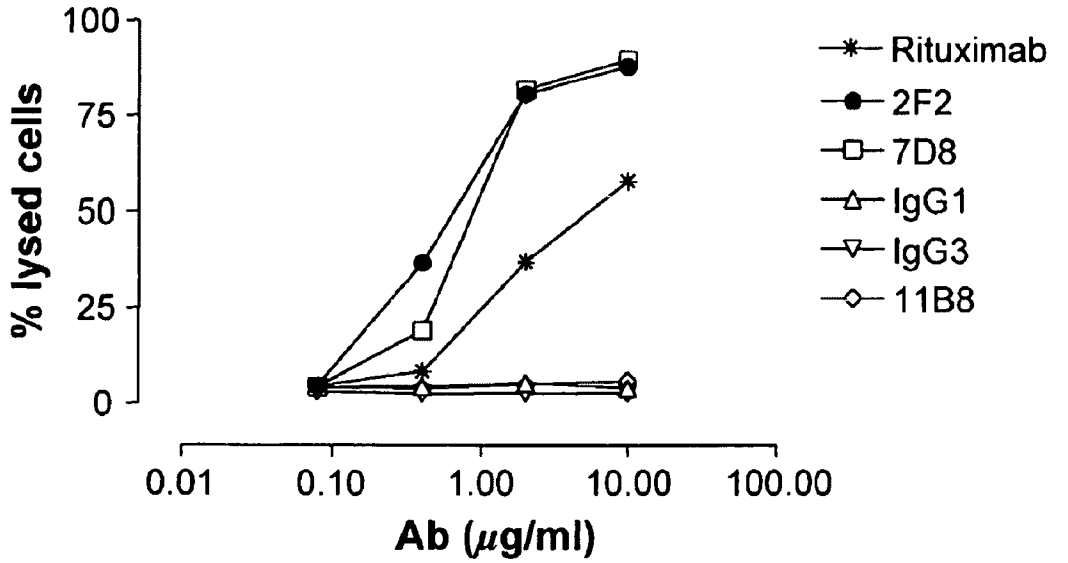


Fig. 14B



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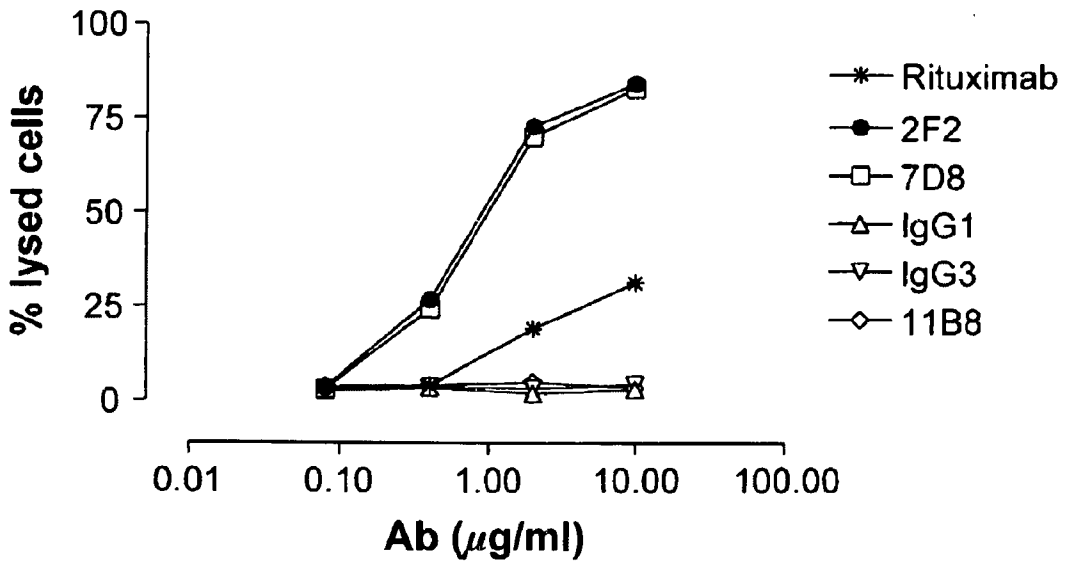
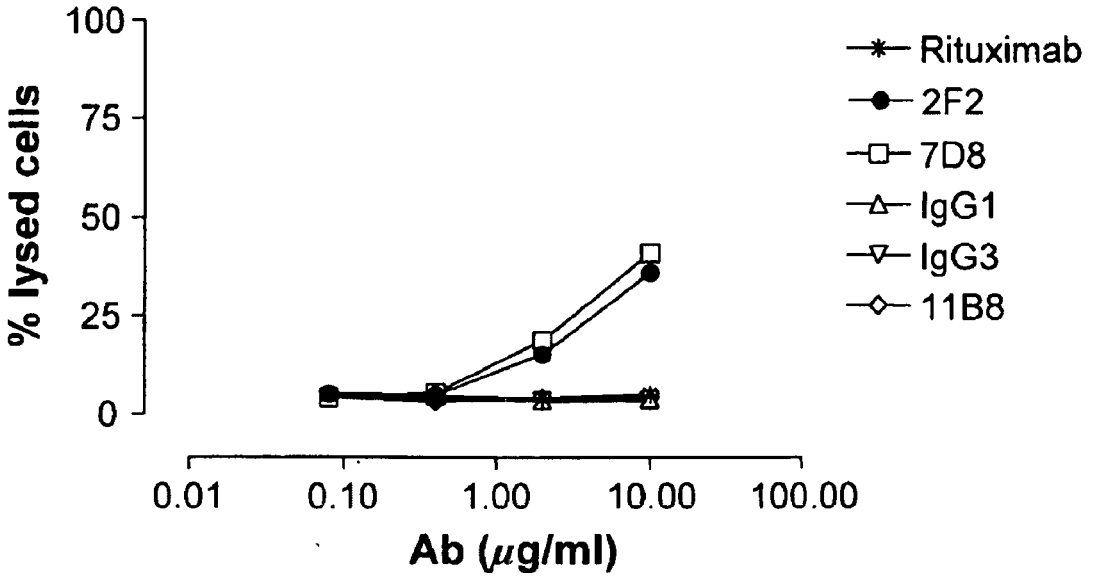


Fig. 15B



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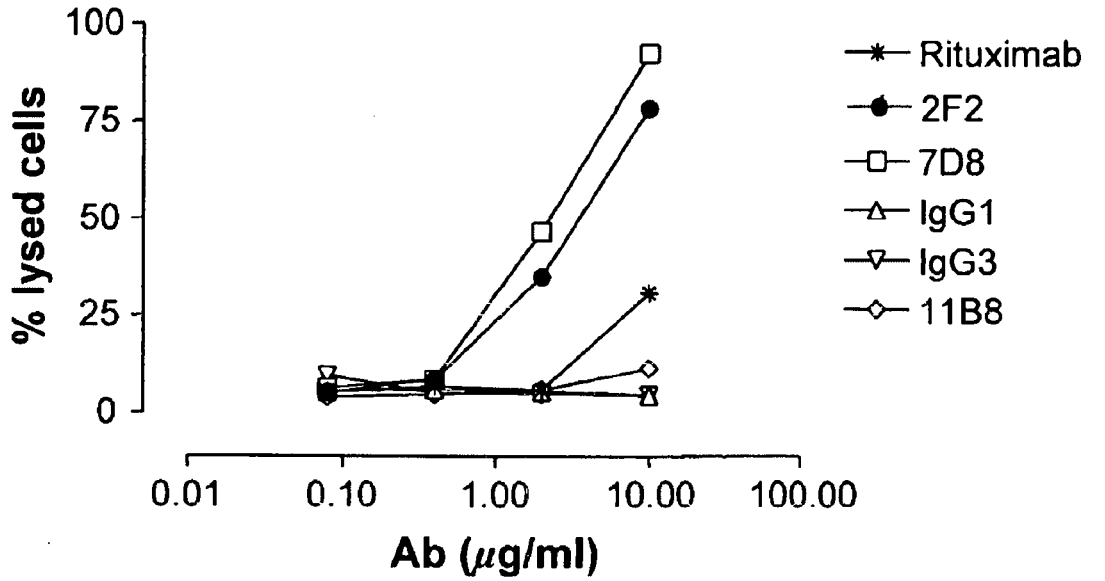

Fig. 16B

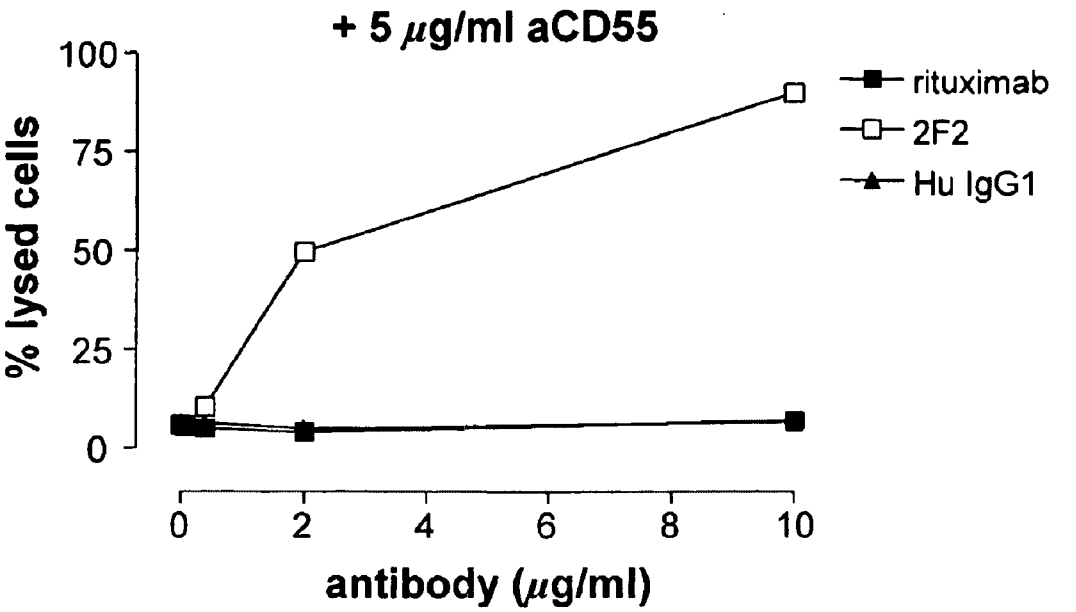

Fig. 17A

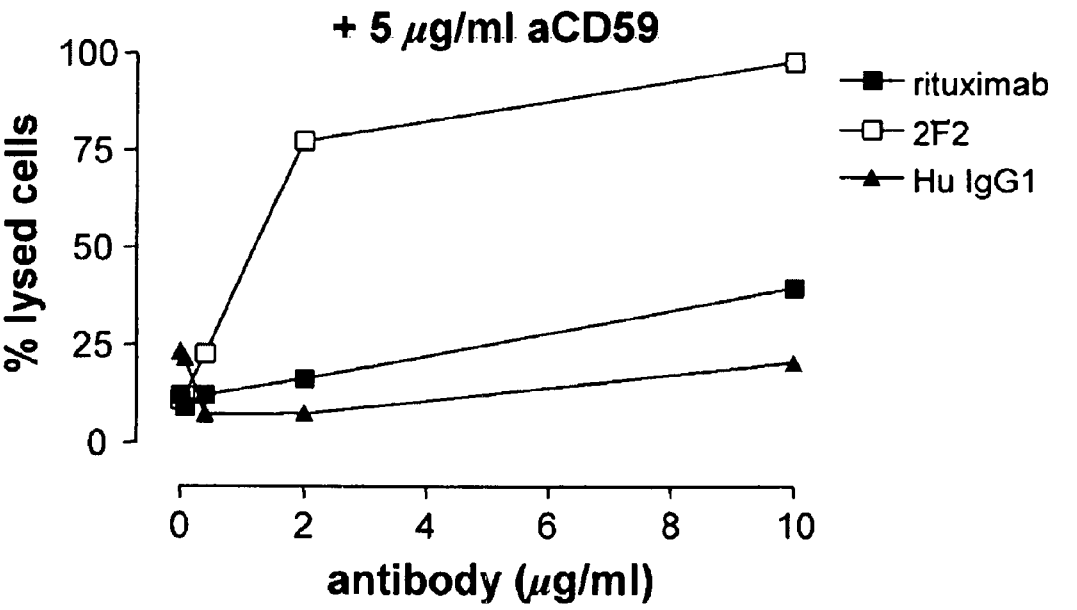

Fig. 17B

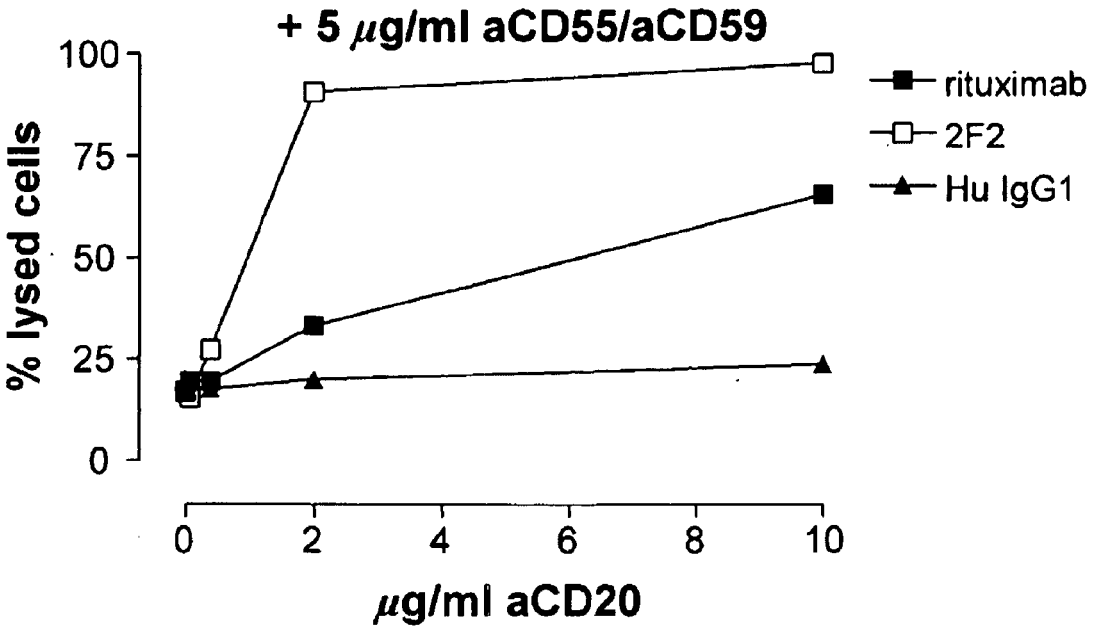
+ 5 μg/ml aCD55/aCD59
% lysed cells
100
75
50
25
0
0
2
4
6
8
10
μg/ml aCD20
rituximab
2F2
Hu IgG1

Fig. 17C

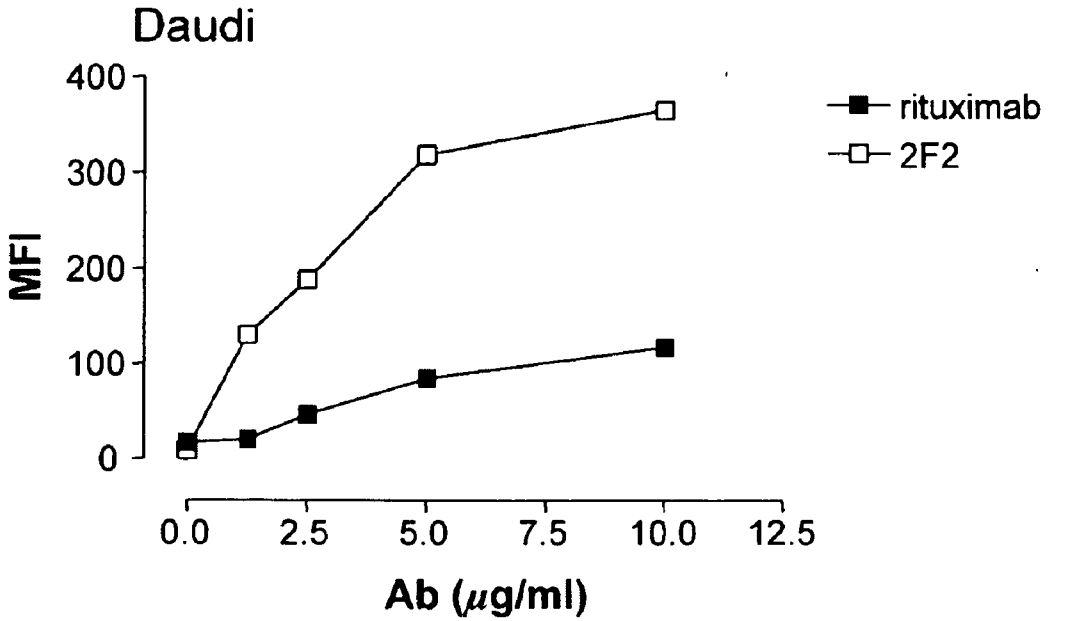

Fig. 18A

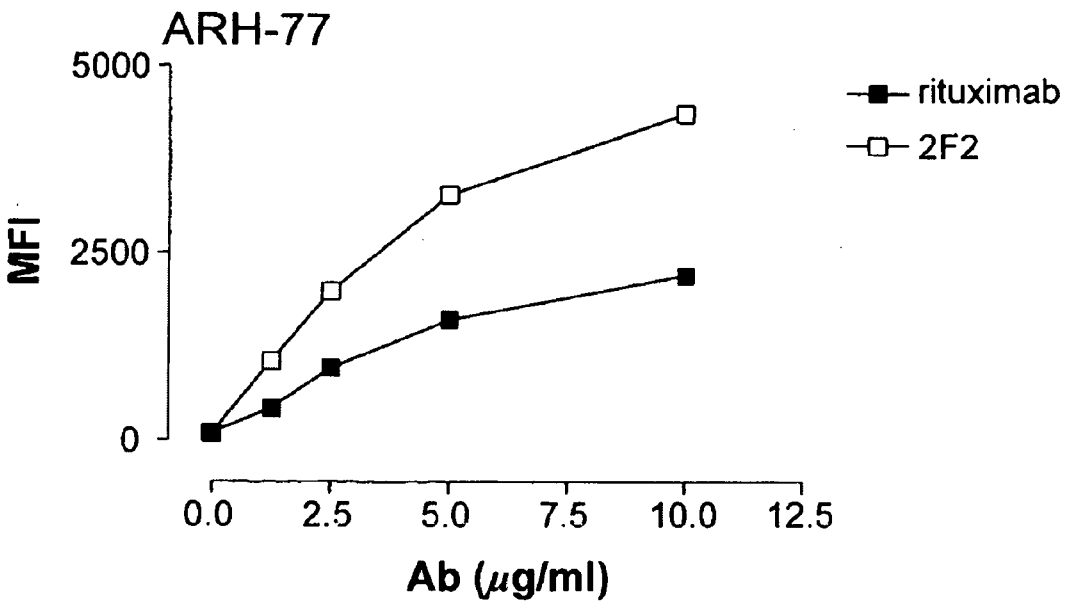

Fig. 18B

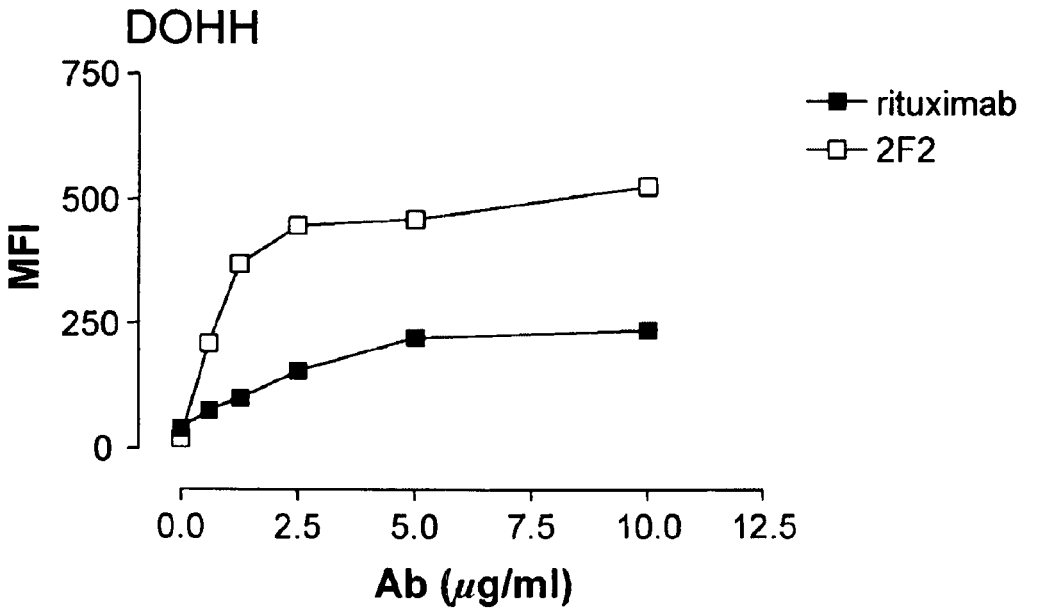

Fig. 18C

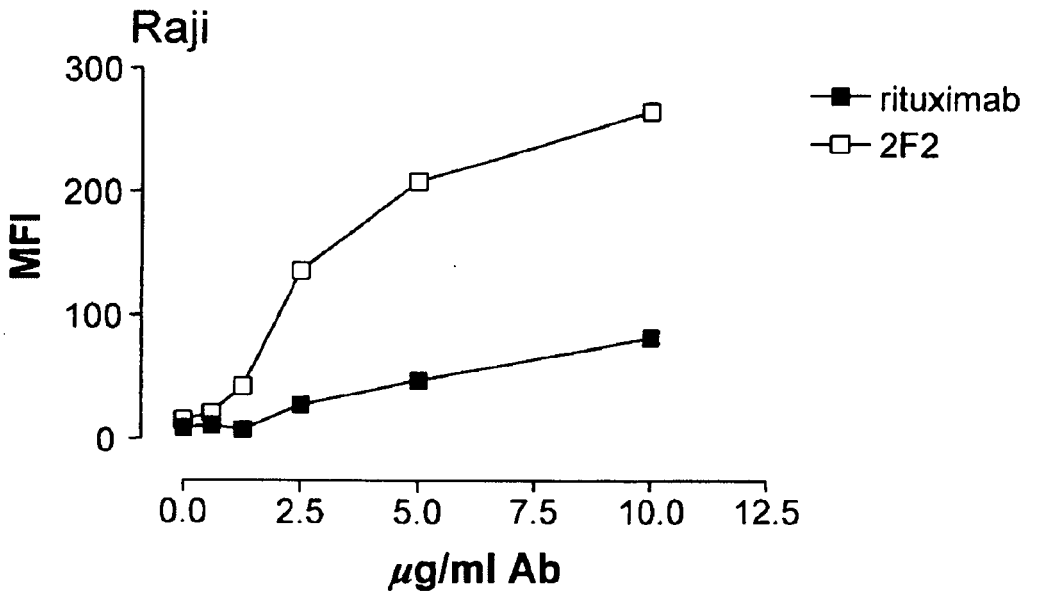

Fig. 18D

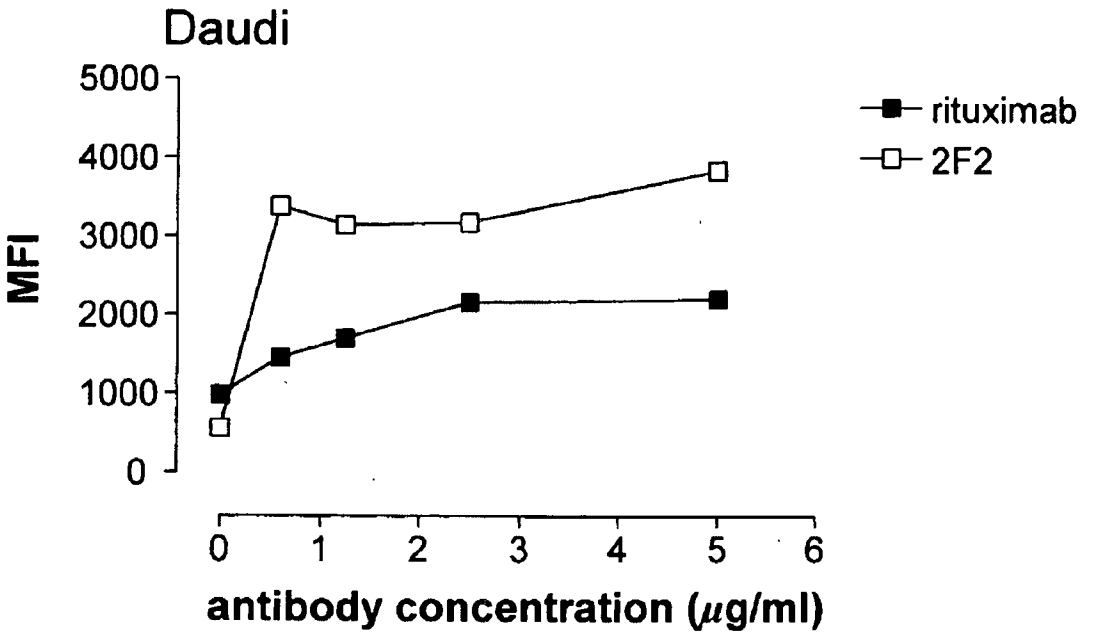

Fig. 19A

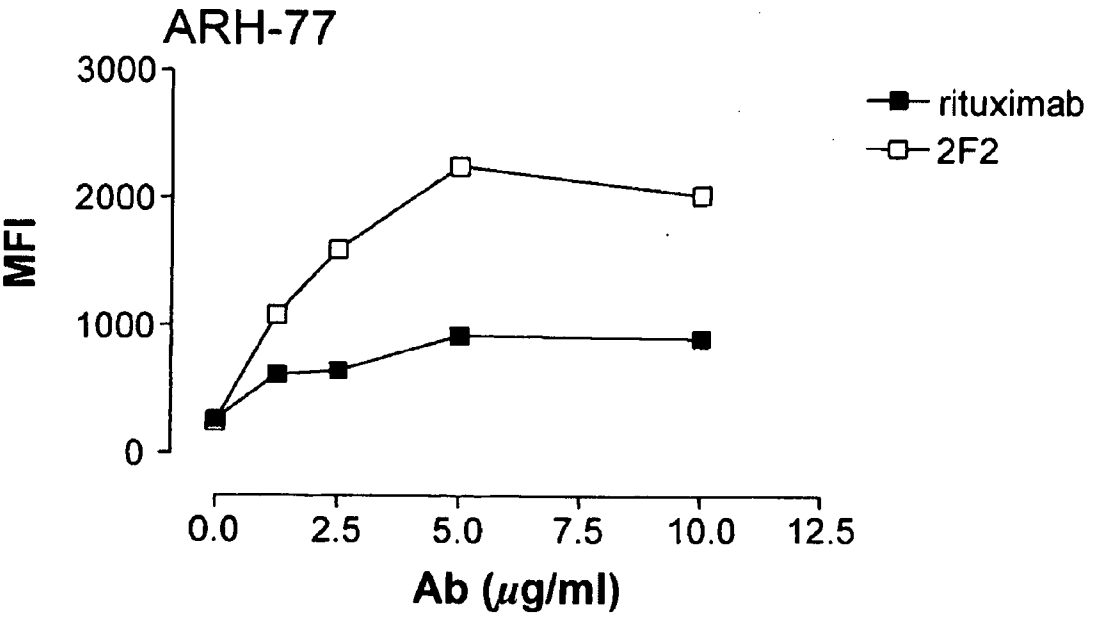

Fig. 19B

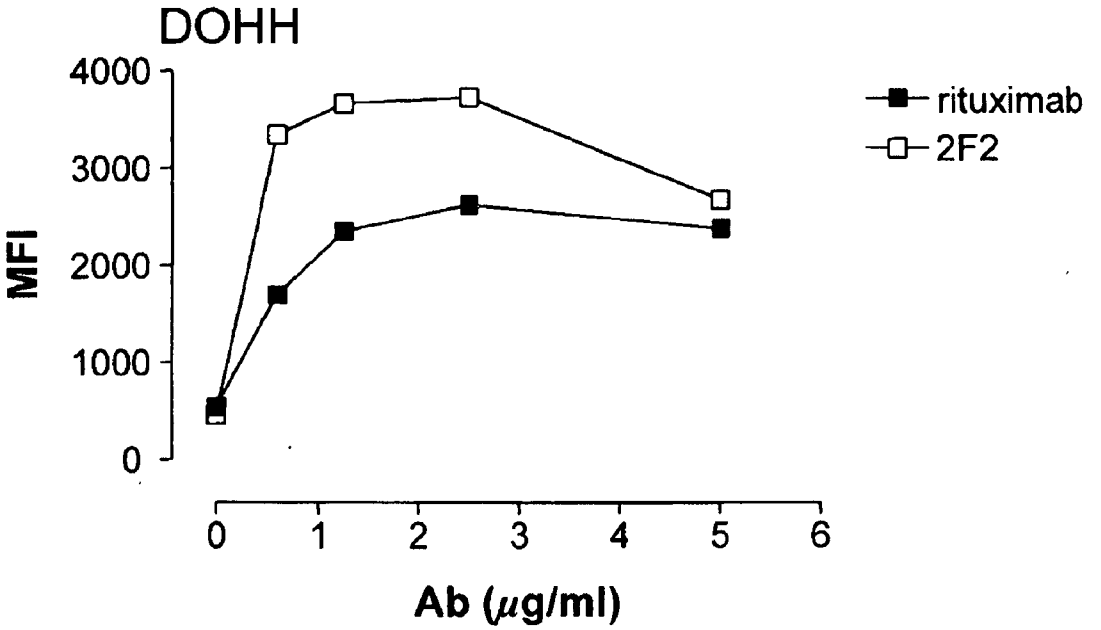

Fig. 19C

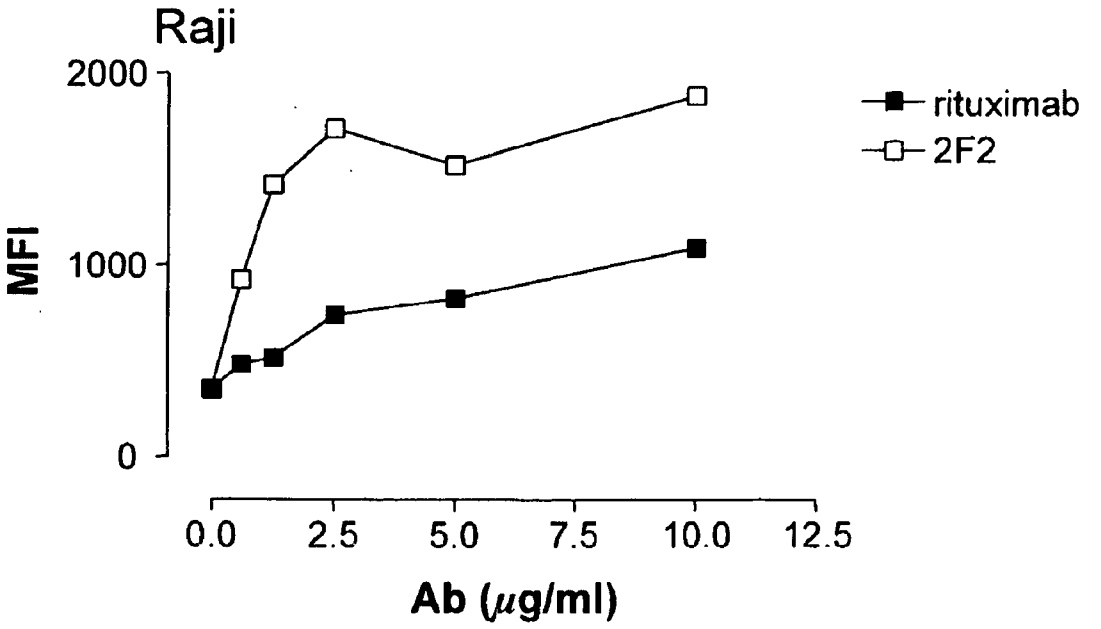

Fig. 19D

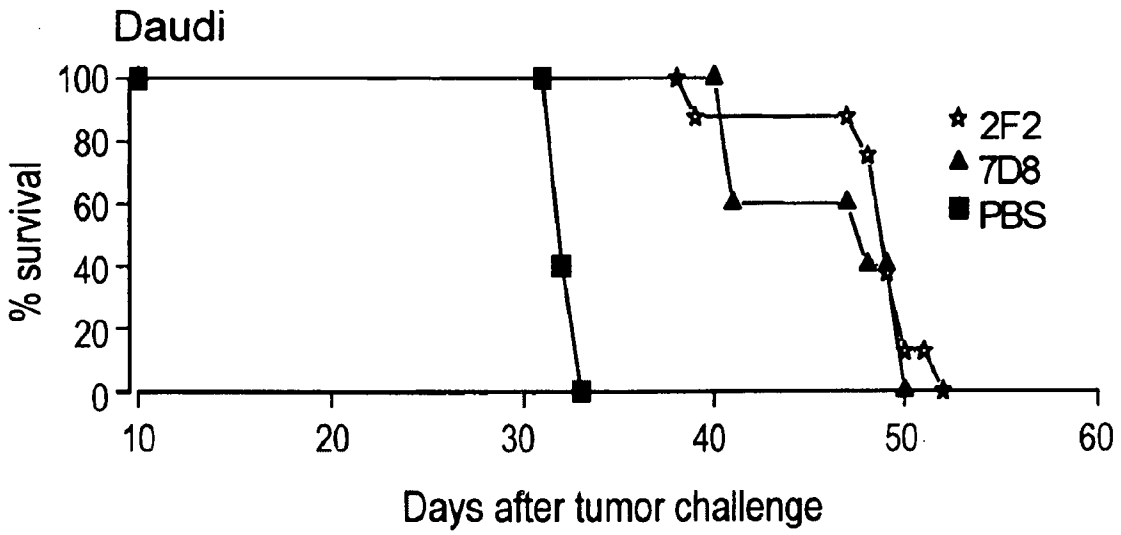
Daudi
100
80
60
40
20
0
% survival
10
20
30
40
50
60
Days after tumor challenge
2F2
7D8
PBS

Fig. 38

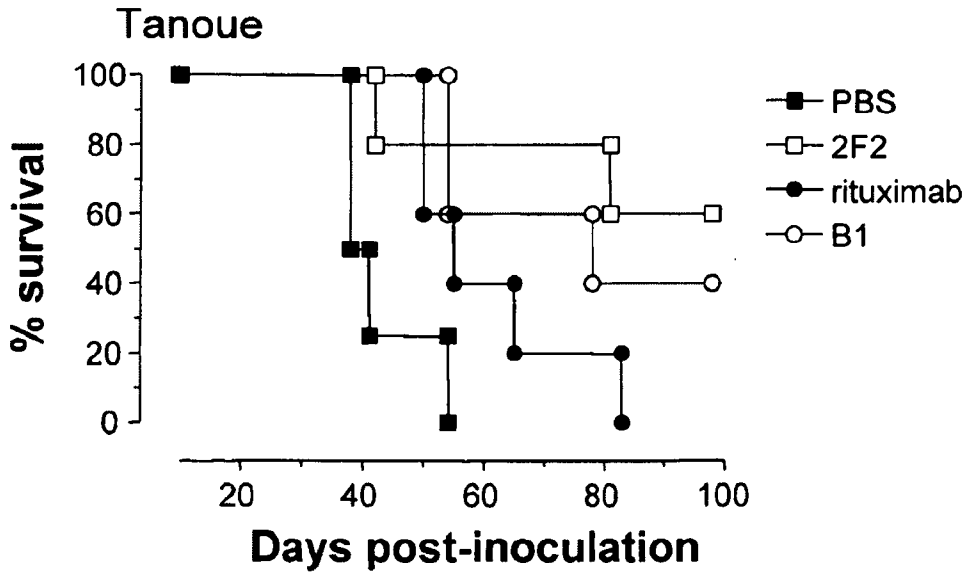
Tanoue
100
80
60
40
20
0
% survival
20
40
60
80
100
Days post-inoculation
PBS
2F2
rituximab
B1

Fig. 39

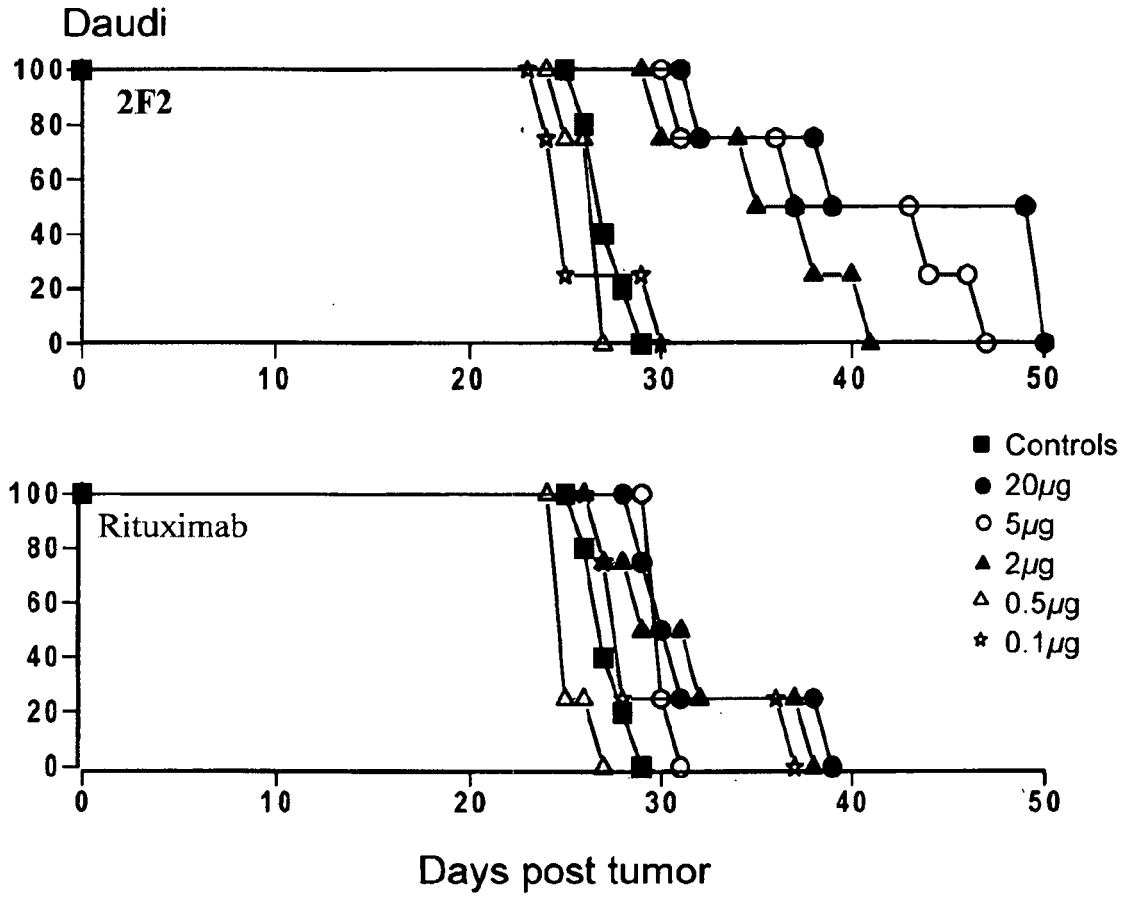
Daudi
2F2
Rituximab
100
80
60
40
20
0
0
10
20
30
40
50
Controls
20μg
5μg
2μg
0.5μg
0.1μg
Days post tumor

Fig. 40

Translation of 2F2 VH

```
  1  MFLGLSWIFL LAILKGVQCE VQLVESGGGL VQPGRSLRLS CAASGFTFND
 51  YAMHWVRQAP GKGLEWVSTI SWNSGSIGYA DSVKGRFTIS RDNAKKSLYL
101  QMNSLRAEDT ALYYCAKDIQ YGNYYYGMDV WGQGTTVTVS S
```

Translation of 2F2VL

```
  1  MEAPAQLLFL LLLWLPDTTG EIVLTQSPAT LSLSPGERAT LSCRASQSVS
 51  SYLAWYQQKP GQAPRLLIYD ASNRATGIPA RFSGSGSGTD FTLTISSLEP
101  EDFAVYYCQQ RSNWPITFGQ GTRLEIK
```

CDR1

CDR2

CDR3

Fig. 53

present in each well. Cell supernatants from 436 transfectants were screened for assembled antibody by IgG,κ-ELISA. Using this data, 111 transfectants were selected for progression and further assessment in static culture. Cultures of the selected cell lines were expanded and adapted to low-serum containing medium (containing bovine serum albumin (BSA) and added 1% dFCS) and a further assessment of productivity in static culture was undertaken (ELISA and measurement of percentage confluence). The 65 highest ranking cell lines were selected for progression. A preliminary assessment of the productivity of the selected cell lines was made in batch shake flask suspension culture in low serum-containing medium (containing BSA and added 1% dFCS). Based upon harvest antibody concentration (by ELISA) and acceptable growth characteristics, 30 cell lines were selected for further evaluation in serum-free medium using a batch shake flask suspension culture. The 10 cell lines that produced the highest antibody concentrations were further evaluated in duplicate fed-batch shake flask suspension cultures in serum-free medium. Product concentrations at harvest were determined by protein A high performance liquid chromatography (HPLC), according to well-known standard methods. All cell lines produced 2F2 antibody (denoted 2F2T) in good yields in the range of from 671-1333 mg/L as determined by protein A HPLC.

[0405] 11B8T: In a similar way a GS-NS/0 cell line was established for recombinant production of 11B8 (denoted 11B8T) modifying the transfection procedure slightly as follows.

[0406] Four transfections of NS/0 host cells were performed, by electroporation with plasmid DNA, using the above linear DNA plasmid. After examining the resulting colonies microscopically to verify that the colonies were of a suitable size for assay (covering greater than 60% of the bottom of the well) and that only one colony was present in each well, cell supernatants from 596 transfectants were screened for assembled antibody by IgG,κ-ELISA. Using this data, 100 transfectants were selected for progression and further assessment in static culture. Cultures of the selected cell lines were expanded and adapted to low-serum containing medium (containing bovine serum albumin (BSA) and added 1% dFCS) and a further assessment of productivity in static culture was undertaken (ELISA and measurement of percentage confluence). The 60 highest ranking cell lines were selected for progression, and an additional 13 cell lines for which productivity data was unavailable were also progressed. A preliminary assessment of the productivity of the selected cell lines was made in batch shake flask suspension culture in low serum-containing medium (containing BSA and added 1% dFCS). Based upon harvest antibody concentration (by ELISA) and acceptable growth characteristics, 10 cell lines were selected for further evaluation in duplicate fed-batch shake flask suspension cultures in low serum-containing medium (containing BSA and added 1% dFCS). Product concentrations at harvest were determined by protein A high performance liquid chromatography (HPLC), according to well-known standard methods. Based on this one of the cell lines was discarded. The resulting 9 cell lines all produced 11B8 antibody (denoted "11B8T") in good yields in the range of from 354-771 mg/L as determined by protein A HPLC.

Example 4

Comparison of Hybridoma-derived 2F2 and Transfectoma-derived Recombinant 2F2T

[0407] By use of gel electrophoresis (SDS-PAGE and native agarose gel electrophoresis) it was shown that 2F2 and 2F2T are of the same size, and only slightly differ in electric charge.

[0408] Furthermore, 2F2 and 2F2T bind to CD20-transfected NS/0 cells and Raji cells with similar affinity as measured by flow cytometry using FACScalibur™ (Becton Dickinson, San Diego, Calif., USA). No binding to non-transfected NS/0 cells was observed demonstrating the specificity of 2F2 and 2F2T. 2F2 and 2F2T also induce CDC in a concentration-dependent manner to the same extent in APH-77 cells (IgG plasma cell leukemia), Daudi cells, DOHH cells (refractory immunoblastic B cell lymphoma progressed from follicular centroblastic/centrolytic lymphoma, DSMZ, Braunschweig, Germany) and Raji cells, measuring cell lysis (number of PI-positive cells) by flow cytometry (FACScalibur). In a second experiment, the concentrations of 2F2 and 2F2T were kept constant while serum was added in different concentrations. No significant differences between 2F2 and 2F2T were observed.

[0409] Finally, 2F2 and 2F2T bound to cell-associated CD20 binds complement factor C1q strongly and to the same extent. The experiment was performed in Daudi cells, DOHH cells, and Raji cells using fluorescein-conjugated anti-C1q polyclonal antibodies for detecting binding of C1q.

Example 5

Binding Characteristics of Human Antibodies Against CD20

[0410] Binding to different cell lines: NS/0, NS/0 transfected with human CD20, Daudi and Raji cells were incubated for 30 min at 4° C. with culture supernatant containing human antibodies 2F2, 7D8, and 11B8 followed by incubation with FITC-conjugated anti-human IgG Ab. Binding was assessed by flow cytometry using a FACScalibur flow cytometer. Fluorescence intensities were compared with negative control isotype matched samples. As shown in **FIG. 4**, all three antibodies bound to NS/0 cells transfected with human CD20, whereas no binding was observed to parental, non-transfected NS/0 cells. All three antibodies also bound to the two different Burkitt lymphoma B cell lines (Raji and Daudi) indicating that 2F2, 7D8, and 11B8 are CD20 specific. Supernatant containing 7D8 or 11B8 were tested under non-saturating conditions, therefore, lower mean fluorescence intensities compared to 2F2 are observed.

[0411] EC_{50} value of 2F2 as determined by flow cytometry: In order to determine the apparent affinity of 2F2 for CD20 expressed on human B cells, a binding curve was made of 2F2 using isolated PBMCs from three human donors and gating of CD3-negative cells. The isolated PBMCs were incubated for 1 hour with a concentration range of FITC labeled 2F2 and analysed on FACS, and the mean fluorescence intensity (MFI) determined. The MFI values are shown in **FIGS. 5A and 5B** as a function of the antibody concentration. The EC_{50} values were calculated by use of Graph Pad Prism 3.02 by non-linear regression. The

EC_{50} value of 2F2 in humans was similar for all three donors with a mean (±s.e.m.) of 287±12.7 ng/ml (1.9±0.1 nM).

[0412] Binding of ^{125}I-labeled mAbs to CD20 expressing cells: mAbs were iodinated using Iodobeads (Pierce Chemical Co., Rockford, Ill.). ^{125}I-labelled mAbs were serially diluted and incubated with Ramos-EHRB cells for 2 hours at 37° C. in the presence of sodium azide and 2-deoxyglucose to prevent endocytosis. The cell bound and free ^{125}I labeled mAbs were then separated by centrifugation at 14,000×g for 2 min through a mixture of phthalate oils, allowing rapid separation without disturbing the binding equilibrium. The pelleted cells together with bound antibody were then counted using a gamma counter (Wallac UK Ltd, Milton Keynes, UK).

[0413] As shown in **FIG. 6**, 2F2 and 11B8 exhibit similar K_D (or similar saturation points) indicating that both antibodies bind with similar affinity. However, 11B8 saturates at a lower level than 2F2 indicating that it recognized a different form of CD20. This is also in agreement with a further experiment showing that a similar number of 2F2 and rituximab antibody molecules binds to CD20 on Ramos-EHRB cells and Daudi cells, as shown by the similar levels of binding saturation (approximately 2-3×10^5 antibody molecules per cell). 11B8 and B1, in contrast, saturate at half this level and only about 1-2×10^5 antibody molecules bind to the Ramos-EHRB cells (**FIG. 7A**) and Daudi cells (**FIG. 7B**).

[0414] To exclude the possibility that the iodinated antibodies bind via Fc-receptors, binding curves were confirmed by use of anti-CD20 $F(ab')_2$ fragments. Again, similar numbers of 2F2 and rituximab-$F(ab')_2$ fragments bound to both Ramos-EHRB and Daudi cells. Also in these experiments, the number of 2F2 or rituximab antibody molecules bound to Ramos-EHRB cells and Daudi cells saturates at approximately twice the number of 11B8 and B1 molecules bound to the cells.

[0415] Dissociation rate: To determine the dissociation rate of the mAbs, Ramos-EHRB cells (final volume of 1 ml in the presence of azide/2DOG) were incubated for 2 hours at 37° C. with 2 μg/ml ^{125}I mAbs to achieve maximum binding. Following centrifugation in a microfuge (2000 rpm for 2 min), the supernatant was removed, the pellet quickly resuspended in 1 ml medium, and immediately transferred to 9 ml medium at 37° C. in a 15 ml conical tube. At various times over the next 2 hours, 0.4 ml samples were removed and separated on phthalate oils to determine the level of radiolabeled mAbs remaining on the cell surface. As shown in **FIG. 8**, both 2F2 and 11B8 dissociated significantly more slowly from CD20 than rituximab or B1.

[0416] Dissociation rates of anti-CD20 $F(ab)_2$ fragments: Ramos-EHRB cells were saturated with 2 μg/ml of ^{125}I-labeled $F(ab)_2$ fragments of 2F2, 11B8, and rituximab, respectively. The Ramos-EHRB cells were washed and incubated in the presence of a high concentration of the unlabeled antibody. The maximal (initial) binding to Ramos-EHRB cells was set at 100%. At several time points over the next 3 hours following loading, 0.4 ml samples were removed and separated on phthalate oil to determine the levels of radiolabeled mAb remaining on the cell surface. As can be seen from **FIG. 9**, 2F2 and 11B8 dissociated much more slowly from the surface of CD20 than rituximab. At 90 min, approximately 50% of the $F(ab)_2$ rituximab molecules were bound to the cell, whereas half of the $F(ab)_2$ 2F2 molecules were dissociated after 3 hours. The k_d (t_{off}) values for 2F2, 11B8T, and rituximab are calculated as follows:

$F(ab)_2$ 2F2: $k_d=\ln 2/t_{1/2}(sec)=\ln 2/10800(sec)=6.4\times 10^{-5}\ sec^{-1}$

$F(ab)_2$ 11B8T: $k_d=\ln 2/t_{1/2}(sec)=\ln 2/9000(sec)=7.7\times 10^{-5}\ sec^{-1}$

$F(ab)_2$ rituximab: $k_d=\ln 2/t_{1/2}(sec)=\ln 2/5400(sec)=1.3\times 10^{-4}\ sec^{-1}$

[0417] Anti-CD20 mAb functional off rates: The impact of the slow 2F2 dissociation rate compared to rituximab was assessed in a functional CDC assay. To this end, Daudi or SU-DHL4 cells were pre-incubated with 10 μg/ml anti-CD20 mAb or an isotype control antibody, washed and incubated in medium for different time points. At these time points after start of the assay, samples were incubated with complement (normal human serum 20 vol/vol %) and then incubated for another 45 min at 37° C. Thereafter, cell lysis was determined on FACS by using PI (propidium iodide) staining method. The % lysed cells (PI-positive cells) are shown in **FIG. 10A** (Daudi cells) or **FIG. 10B** (SU-DHL4 cells) as a function of incubation time. 2F2 induced high CDC in both cell lines, and still lysed up to 90% of the cells after 6 hours, indicating that the CD20 saturation of the cells remained sufficiently high to induce complement-mediated lysis of most of the cells. Rituximab, in contrast and in agreement with the above dissociation rate studies, dissociated rapidly from the cells and failed to induce specific lysis following the 6 hour incubation period. 11B8 was used as a control and did not induce CDC.

Example 6

CDC of Human Antibodies Against CD20

[0418] Serum preparation: Serum for complement lysis was prepared by drawing blood from healthy volunteers into autosep gel and clot activator vacutainer tubes (BD biosciences, Rutherford, N.J.) which were held at room temperature for 30-60 min and then centrifuged at 3000 rpm for 5 min. Serum was harvested and stored at −80° C.

[0419] Flow cytometry: For flow cytometry a FACScalibur flow cytometer was used with CellQuest pro software (BD Biosciences, Mountain view, Calif.). At least 5000 events were collected for analysis with cell debris excluded by adjustment of the forward sideward scatter (FCS) threshold.

[0420] CDC kinetics: In a first set of experiments (n=3) the kinetics of CDC of five different B-cell lines, i.e., Daudi, SU-DHL-4, Raji, DOHH and ARH-77, were determined by adding 10 μg/ml 2F2, rituximab and an IgG control antibody, respectively, for 10 min before human serum was added. At several time intervals (up to one hour) after induction of CDC, the cells were suspended in PI solution and cell lysis (number of PI-positive cells) was measured by flow cytometry. The results are depicted in **FIGS. 11A** (ARH-77 cells), **11B** (Daudi cells), **11C** (Raji cells), **11D** (DOHH) and **11E** (SU-DHL-4). As seen, addition of antibodies induced cell lysis within 5 min. Interestingly, addition of 2F2 resulted in a marked cell lysis of more than 80% in all five B-cell lines. Rituximab induced more than 80% cell lysis only in the SU-DHL-4 and Daudi cell lines, whereas the cell lysis of the DOHH cell line was ~50%, and

less than 20% in the ARH-77 and Raji cell lines. No lysis was observed with the IgG control antibody (data only shown in **FIG. 11B**).

[0421] CDC serum titration: In a separate set of experiments (n=5), NHS (normal human serum) was titrated at two different antibody concentrations of 0.5 μg/ml and 5 μg/ml. Cells were pre-incubated with 2F2 or rituximab for 10 min, before a concentration range of NHS was added. At 45 min after induction of CDC, cells were resuspended in PI solution. Cell lysis (number of PI-positive cells) was measured by flow cytometry. FIGS. **12A-D** show the percentage of lysed (PI-positive) cells as a function of NHS concentration. **FIG. 12A** shows cell lysis of Daudi cells, **FIG. 12B** cell lysis of ARH-77 cells, **FIG. 12C** cell lysis of DOHH cells, and **FIG. 12D** cell lysis of Raji cells. Increased lysis of cells was observed with increased NHS concentration. Addition of 2F2 caused maximal lysis of Daudi cells at the highest NHS and antibody concentration. Rituximab induced about 50% cell lysis of Daudi cells at the highest NHS concentration.

[0422] In ARH-77 cells, only the highest concentration of NHS and 2F2 led to approximately 75% cell lysis. Lower antibody concentrations were insufficient to induce ARH-77 cell lysis. Rituximab was not able to induce cell lysis of ARH-77 cells in this experiment.

[0423] 2F2 was able to induce NHS-concentration dependent cell lysis of DOHH cells at both the high and the low concentration, whereas rituximab was not able to induce lysis under these conditions.

[0424] Finally, 2F2 induced NHS-concentration-dependent lysis of Raji cells, which was only apparent by use of 5 μg/ml mAb. No lysis was observed with rituximab.

[0425] In these experiments, no lysis was observed with the isotype control antibody (data not shown).

[0426] CDC antibody titration: To measure the ability of the anti-CD20 antibodies to induce CDC at low concentrations, an experiment was performed where the antibodies were titrated (n=6). Various cell lines were pre-incubated with a concentration range of 2F2 and rituximab, respectively, for 10 min before NHS was added. After 45 min incubation at 37° C. (when maximal lysis occurs) the cells were resuspended in PI solution and cell lysis (number of PI-positive cells) was measured by flow cytometry. **FIGS. 13A** (Daudi cells), **13B** (DOHH cells), **13C** (ARH-77 cells), and **13D** (Raji cells) show the percentage of lysed (PI-positive) cells as a function of antibody concentration. Both 2F2 and rituximab induced a concentration-dependent increase in cell lysis. 2F2 induced more than 80% lysis of Daudi cells upon addition of 2 μg/ml, whereas with rituximab this level was not reached even after addition of 10 μg/ml. Furthermore, 2F2 induced more than 80% lysis of DOHH cells at 0.4 μg/ml, whereas minimal lysis was observed with rituximab at this concentration. The maximal lysis of DOHH cells with rituximab (~30% of total cell analyzed) was reached at 10 μg/ml. Induction of lysis of ARH-77 and Raji cells by 2F2 was lower, but still ~70% lysis was reached at an antibody concentration of 10 μg/ml. At its highest concentration, rituximab induced lysis in only ~23% of ARH-77 cells, and in only ~6% of Raji cells.

[0427] In a similar experiment, 2F2, 2F2T, 11B8T, and rituximab were investigated for their ability to induce CDC of Daudi and Raji cell lines, see **FIGS. 14A and 14B**. Also in this experiment more than 80% lysis of Daudi cells was observed with (transfectoma-derived) 2F2T at 10 μg/ml, whereas rituximab reached only to 60% lysis even at 10 μg/ml, cf. **FIG. 14A**. Lysis of Daudi cells with 2F2T was identical to the lysis obtained with hybridoma-derived 2F2.

[0428] Lysis of Raji cells was more difficult, but again both 2F2 and 2F2T induced lysis of Raji cells to a similar extent (**FIG. 14B**). Rituximab was not able to induce CDC of Raji cells which is in agreement with the experiment shown in **FIG. 13D**.

[0429] As can be seen from **FIGS. 14A and 14B** neither Daudi nor Raji cells were susceptible to CDC by 11B8T. B1 induced lysis of Daudi cells, but only to a small extent, and was not able to induce lysis of Raji cells.

[0430] CDC activity of anti-CD20 in Daudi cells: To determine the CDC activity of each antibody, elevated membrane permeability was assessed using FACS analysis of propidium iodide (PI)-stained cells. Briefly, the Daudi cells were washed and resuspended in RPMI/1% BSA at 1×10^6 cells/ml. Various concentrations of human monoclonal antibodies were added to the Daudi cells and allowed to bind to CD20 on the cells for 10-15 min at room temperature. Thereafter, serum as a source of complement was added to a final concentration of 20% (v/v) and the mixtures were incubated for 45 min at 37° C. The cells were then kept at 4° C. until analysis. Each sample (150 μl) was then added to 10 μl of PI solution (10 μg/ml in PBS) in a FACS tube. The mixture was assessed immediately by flow cytometry. As shown in **FIG. 15A**, 2F2 and 7D8 showed superior CDC activity compared to rituximab.

[0431] In a second experiment, cells were labeled with human monoclonal antibodies as above, then washed and incubated in PBS for 45 min at 37° C. prior to the addition of human serum. This ensured that only antibody bound to the cell at the time of serum addition was available to activate complement for cell lysis. As shown in **FIG. 15B**, decreased CDC activity was found for rituximab compared to 2F2 and 7D8 indicating that the human anti-bodies (2F2 and 7D8) are not affected by washing the cells prior to the addition of serum.

[0432] CDC activity of anti-CD20 in Raji cells: CDC activity was assessed using Raji cells which have relatively high surface expression of CD55 and CD59 and, therefore, are more resistant to complement attack. Human antibodies were added to Raji cells and allowed to bind for 15 min. Human serum (20%) was added and the mixtures incubated for 45 min at 37° C. As shown in **FIG. 16A**, rituximab was ineffective in mediating CDC of Raji cells whereas significant levels of cell lysis occurred in Raji cells opsonized with 2F2 or 7D8. Accordingly, 2F2 and 7D8 have a unique capacity to lyse CD55/59 positive target cells.

[0433] In a separate experiment, Raji cells were pre-incubated with saturating concentrations of anti-CD55 mAb (final concentration of 5 μg/ml) and anti-CD59 mAb (final concentration of 5 μg/ml) to block the effects of these complement defense molecules. Human anti-CD20 antibodies were then added along with serum (20%) as above for 45 min at 37° C. As shown in **FIG. 16B**, the blockade of CD55 and CD59 molecules resulted in almost 100% lysis of Raji cells with human antibodies 2F2 or 7D8 whereas only a 25% increase in cell lysis was observed using rituximab.

[0434] Role of complement inhibitors I—Expression of surface molecules: Since complement inhibitors such as CD55 and CD59 appear to play an important role in susceptibility to rituximab-induced CDC, an experiment was performed to determine the expression of these molecules on the B-cell lines under investigation (Raji, Daudi, DOHH, ARH-77, and SU-DHL-4).

[0435] The cells were stained with FITC-conjugated anti-CD55, anti-CD59 and anti-CD20 antibodies and molecules expression was analyzed by flow cytometry. The results are shown in the below Table 1.

TABLE 1

Expression	CD20	CD55	CD59
ARH-77	++	++++	++
Raji	+	++	+++
DOHH	++	+++	++
SU-DHL-4	+++	+	++
Daudi	++	+	+

[0436] Role of complement inhibitors II—Blockade of CD55 and CD59: To further study the roles of CD55 and CD59 in anti-CD20-induced CDC, both complement inhibitor molecules were blocked by specific antibodies prior to induction of CDC (n=3). Raji cells were used because only partial lysis was induced by 2F2 alone. Raji cells (1×10^5 cells/50 μl) were pre-incubated with a concentration range of 2F2 and rituximab together with anti-CD55 (5 μg/ml) or anti-CD59 (5 μg/ml) antibodies for 10 min, before pooled NHS (20%) was added. At 45 min after induction of CDC, cells were resuspended in PI solution. Cell lysis (number of PI-positive cells) was measured by flow cytometry. FIGS. **17A-C** show the percentage of lysed (PI-positive) cells as a function of antibody concentration, and show one experiment which is exemplary of three experiments. **FIG. 17A** shows incubation of Raji cells with anti-CD55 antibody, **FIG. 17B** incubation of Raji cells with anti-CD59 antibody, and **FIG. 17C** incubation of Raji cells with anti-CD55 and anti-CD59 antibodies.

[0437] As can be seen in **FIG. 17A**, addition of anti-CD55 antibody did not influence 2F2 or rituximab-induced CDC. Addition of anti-CD59 antibody increased susceptibility of the cells to both 2F2 and to rituximab with ~30% (**FIG. 17B**). Addition of both anti-CD55 and anti-CD59 further enhanced anti-CD20-induced lysis of cells with ~30% (**FIG. 17C**).

[0438] Role of complement factors, as determined by flow cytometry I—C1q binding: Anti-CD20 antibodies (2F2 and rituximab) and an isotype control antibody were added to various B-cell lines. After 10 min incubation, NHS (1 vol/vol %) was added. After further incubation for 10 min at 37° C. and washing of the cells, the supernatant was discarded and the cell pellet was incubated with FITC-conjugated anti-C1q antibody. Data show mean fluorescence intensity of cells stained with C1q and are depicted in **FIGS. 18A** (Daudi), **18B** (ARH-77), **18C** (DOHH), and **18D** (Raji) (n=6). The results indicated antibody concentration-dependent increase in binding of C1q by 2F2, irrespective of the B-cell line investigated. Moreover, C1q binding by 2F2 was always higher than binding by rituximab, in all cell lines tested. No increase in mean fluorescence was observed with the isotype control antibody (data not shown).

[0439] Role of complement factors, as determined by flow cytometry II—Complement activation via the classical route: Fixation of C4c to antibody-coated cells is an indication of activation of complement activation via the classical route. Anti-CD20 antibodies (2F2 and rituximab) and an isotype control antibody were added to various B-cell lines. After 10 min incubation at 37° C., NHS (1 vol/vol %) was added. After further incubation and washing of the cells, the supernatant was discarded and the cell pellet was incubated with FITC-conjugated anti-C4c antibody. Data show mean fluorescence intensity of cells stained with C4c and are depicted in **FIGS. 19A** (Daudi), **19B** (ARH-77), **19C** (DOHH), and **19D** (Raji) (n=6). Complement factor C4c fixation to 2F2 was demonstrated in all B-cell lines tested (n=3), with a maximum reached at ~1 μg/ml of antibody. Fixation of C4c after 2F2 binding was much higher than after rituximab, irrespective of the cell line tested. No increase in mean fluorescence was observed with the isotype control antibody (data not shown).

[0440] CDC in heat-inactivated serum: Cells (Daudi cells, ARH-77 cells or Raji cells) and antibodies (rituximab, 2F2, 2F2T, 11B8, and isotype control antibody HuMab-KLH IgG1) were pre-incubated in a concentration range of anti-CD20 antibodies for 10 min, before NHS (active or heat-inactivated in a water bath at 57° C. at 30 min) was added. At 45 min after induction of CDC, cells were resuspended in PI solution. Cell lysis (number of PI-positive cells) was measured by flow cytometry. No lysis of the cells was observed in the presence of heat-inactivated serum, irrespective of the cell-line and CD20-antibody used, no CDC was observed in the presence of heat-inactivated serum.

Example 7

ADCC of Human Antibodies Against CD20 ADCC Assay I

[0441] Enrichment of human neutrophils: Polymorphonuclear cells (neutrophils, PMNs) were enriched from heparinized whole blood. Blood was diluted twice in RPMI 1640 and was layered on Ficoll (Lymphocyte Separation Medium 1077 g/ml, 710 g, RT, 20 min; BioWhittaker, cat. 17-829E, lot no. 0148 32) and centrifuged at 2000 rpm for 20 min. The mononuclear cell layer was removed, and erythrocytes within the pellet containing neutrophils were hypotonically lysed using ice-cold NH_4Cl solution (155 mM NH_4Cl, 10 mM $NaHCO_3$, 0.1 mM EDTA, pH 7.4). The remaining neutrophils were washed twice and resuspended in RPMI 1640 supplemented with 10% FCS (RPMI-10).

[0442] Enrichment of human peripheral blood mononuclear cells: Human blood was diluted twice in RPMI 1640 and blood cells were layered on Ficoll (Lymphocyte Separation Medium 1077 g/ml, 710 g, RT, 20 min; BioWhittaker, Cambrex Bio Science Verviers, Verviers, Belgium, cat. 17-829E, lot no. 0148 32). Peripheral blood mononuclear cells (MNCs) were collected from the interphase, washed and resuspended in RPMI 1640 culture medium supplemented with 10% FCS, 2 mM L-glutamine, 5 U/ml penicillin, 50 μg/ml streptomycin (all derived from BioWhittaker) to which 25 mM HEPES (BioWhittaker) was added.

[0443] ADCC set up: Target B-cells (freshly isolated B-cells or from B-cell lines) were labeled with 20 μCi ^{51}Cr (Amersham Biosciences, Uppsala, Sweden) for 2 hours.

After extensive washing in RPMI-10, the cells were adjusted to $1..10^5$ cells/ml. Whole blood or isolated effector cells (50 μl; MNCs, PMNs) or plasma (50 μl), sensitizing antibodies (50 μl), and RPMI-10 (50 μl) were added to round-bottom microtiter plates (Greiner Bio-One GmbH, Frickenhausen, Germany). Assays were started by adding target cells (50 μl) giving a final volume of 200 μl. For isolated effector cells, an effector to target (E:T) ratio of 40:1 was used. For whole blood, an amount of 33 vol/vol % was used corresponding to an estimated effector to target ratio of 40:1. After incubation (3 hours, 37° C.), assays were stopped by centrifugation, and ^{51}Cr release from triplicates was measured in counts per minute (cpm) in a scintillation counter. Percentage of cellular cytotoxicity was calculated using the following formula:

% specific lysis=(experimental cpm−basal cpm)/(maximal cpm−basal cpm)×100

[0444] with maximal ^{51}Cr release determined by adding perchloric acid (3% final concentration) to target cells, and basal release measured in the absence of sensitizing antibodies and effector cells.

[0445] Statistics: Data were analyzed by one-way ANOVA, followed by Tukey's multi comparison post-hoc test. Analysis was performed using Graph Pad Prism (version 3.02 for Windows, Graph Pad Software, San Diego, Calif., USA).

[0446] Lysis of ARH-77 cells: In a first set of experiments, ARH-77 cells were used as target cells (**FIG. 20**). Addition of 2F2 (n=3), rituximab (n=3) or 11B8T (n=1) resulted in MNC-mediated lysis of ARH-77 cells of approximately 50%. No specific lysis was observed in the presence of neutrophils. Addition of plasma (to evaluate the role of complement) induced lysis of ARH-77 cells after incubation with 2F2, but not after incubation with rituximab ($p<0.05$, 2F2 vs. no antibody, ANOVA) or 11B8T. In the presence of whole blood, lysis of ARH-77 cells increased after incubation with 2F2 ($p<0.05$, 2F2 vs. rituximab and 2F2 vs. no antibody, ANOVA), but not with rituximab. Specific lysis induced by rituximab was in fact very low in the presence of whole blood. 11B8T induced cell lysis of approximately 25% (n=1) in the presence of whole blood. In the absence of antibody non-specific lysis of 10-15% was observed.

[0447] Lysis of B-CLL cells: In a second set of experiments, chronic B-lymphocytic leukaemia (B-CLL) cells obtained from B-CLL patients (n=12) were subcloned for 5 rounds and then used as target cells in the experiment (**FIG. 21**). In the absence of antibody, no specific lysis was observed, but addition of 2F2, 11B8T or rituximab (10 μg/ml) increased MNC-mediated specific lysis to 10-20% ($p<0.001$, ANOVA). Incubation of target cells with plasma and 2F2 induced specific lysis of B-CLL cells, whereas no specific lysis was observed with 11B8T or rituximab ($p<0.001$, ANOVA). Moreover, 2F2 mediated specific lysis of B-CLL cells after incubation in whole blood. No specific lysis of B-CLL cells by whole blood was observed with 11B8T ($p<0.01$, ANOVA) or rituximab ($p<0.001$, ANOVA). No specific lysis was observed in the presence of neutrophils.

[0448] Because rituximab was able to mediate effective ADCC but not CDC of the tumor cells tested, it is likely that whole blood-induced B-cell lysis by 2F2 is mediated via complement.

[0449] Lysis of hairy cell leukaemia (HCL) cells: In a third set of experiments lysis of HCL cells by 2F2, 11B8T, and rituximab by ADCC or in the presence of plasma or whole blood was determined. Data are shown in **FIG. 22**. Whereas neutrophils could not mediate ADCC irrespective of the mAb used, 11B8T was able to induce MNC-mediated lysis of HCL cells more efficiently than 2F2 ($p<0.001$, ANOVA) or rituximab ($p<0.05$, ANOVA). 2F2 and rituximab were not able to induce MNC-mediated lysis of HCL cells. Plasma-mediated lysis of the cells was strongly enhanced with 2F2, as compared to rituximab ($p<0.05$, ANOVA), 11B8T ($p<0.01$, ANOVA) or without antibody ($p<0.001$, ANOVA). When lysis induced by anti-CD20 in the presence of whole blood was studied, 2F2 induced complete lysis of cells, and was superior to rituximab ($p<0.01$, ANOVA), 11B8T or no antibody added ($p<0.001$, ANOVA).

[0450] Lysis of B-ALL cells: Using cells from two patients the ability of 2F2 and rituximab to induce lysis B-ALL cells by ADCC or complement was investigated (**FIG. 23**). As was observed in the previous experiments, 2F2 and rituximab induced MNC-mediated ADCC of B-ALL cells to a similar extent. But again 2F2 was able to induce plasma- and whole blood-mediated lysis of B-ALL cells, whereas rituximab was not.

[0451] Lysis of follicular lymphoma cells: When lysis of follicular lymphoma cells (n=2) was investigated, a different picture emerged (**FIG. 24**). A minor PMN-mediated lysis of cells with 2F2 was observed, and both 2F2 and rituximab were not able to induce MNC-mediated ADCC. 11B8T was still able to induce MNC-mediated lysis of approximately 20%. Although a relatively high plasma-mediated lysis was induced by rituximab, complete plasma-mediated lysis was observed with 2F2. Also with whole blood, complete lysis was observed with 2F2, whereas 70% lysis with rituximab. Minimal plasma- or whole blood-mediated lysis by 11B8T was observed.

[0452] Lysis of primary mantle cell lymphoma cells: Specific lysis of mantle cell lymphoma cells was more difficult to induce (n=1, **FIG. 25**). Minimal or no lysis by 2F2, 11B8T or rituximab was observed after addition of PMN or MNC and CD20 mAbs. However, 2F2 was still able to induce approximately 40% lysis by plasma or whole blood, whereas with rituximab only 10-20% of the cells were lysed. 11B8T was not able to induce lysis of primary mantle cell lymphoma cells.

[0453] Antibody concentration-dependent lysis of ARH-77 cells in whole blood: In a further experiment (n=4) dose-dependency regarding the induction of ADCC on ARH-77 cells in the presence of whole blood was analyzed. As can be seen in **FIG. 26**, titration of 2F2 induced a dose-dependent increase in the percentage of specific lysis ($p<0.05$: treatment-effect, two-way ANOVA) of ARH-77 cells. No specific lysis of ARH-77 cells was observed with rituximab.

ADCC Assay II

[0454] Preparation ^{51}Cr-labeled target cells: ARH-77 cells and Raji cells were collected ($3..10^6$ cells) in RPMI++, spun down (1500 rpm; 5 min), resuspended in 140 μl ^{51}Cr (Chromium-51; CJS11-1 mCi, batch 12; 140 μl is about 100 μCi) and incubated (37° C. water bath; 1 hour). After washing cells (1500 rpm, 5 min, in PBS, 3×), cells were

resuspended in RPMI++ and counted by trypan blue exclusion. Cells were brought at concentration of 2×10^4 cells/ml.

[0455] Preparation of effector cells: Fresh peripheral blood mononuclear cells (MNC) were isolated from 40 ml of heparin blood by Ficoll (Bio Whittaker; lymphocyte separation medium, cat 17-829E) via manufacturer's instructions. After resuspension of cells in RPMI++, cells were counted by trypan blue exclusion and adjusted to a concentration of 1×10^6 cells/ml.

[0456] ADCC set up: 50 μl RPMI++ was pipetted into 96 wells plates, and 50 μl of ^{51}Cr-labeled target cells were added. Thereafter, 50 μl of antibody was added, diluted in RPMI++ (final concentrations 10, 1, 0.1, 0.01 μg/ml). Cells were incubated (RT, 10 min), and 50 μl effector cells were added, resulting in an effector to target ratio of 50:1 (for determination of maximal lysis, 50 μl 5% Triton-X-100 was added instead of effector cells). Cells were spun down (500 rpm, 5 min), and incubated:(37° C., 5% CO_2, 4 hours). After spinning down the cells (1500 rpm, 5 min), 100 μl of supernatant was harvested into micronic tubes, and counted in a gamma counter. The percentage specific lysis was calculated as follows:

% specific lysis=(cpm sample−cpm target cells only)/(cpm maximal lysis−cpm target cells only)×100

[0457] Statistics: Data were analyzed by one-way ANOVA, followed by Tukey's multi comparison post-hoc test. Analysis was performed using Graph Pad Prism (version 3.02 for Windows, Graph Pad Software, San Diego, Calif., USA).

[0458] Antibody concentration-dependent lysis of ARH-77 and Raji cells: 2F2T and 11B8T were tested for their ability to induce ADCC of ARH-77 and Raji cells (n=3) in comparison with rituximab.

[0459] A dose-effect relation with CD20 mAbs was observed in ADCC of ARH-77 cells using MNC as effector cells (**FIG. 27**). Both 2F2T and 11B8T induced specific lysis of ARH-77 cells which was maximal (50%) at 10 μg/ml of mAb. Rituximab induced only 25% lysis of target cells. Addition of the isotype control antibody (HuMab-KLH) did not induce ADCC. No specific lysis was observed without addition of MNCs (data not shown).

[0460] When Raji cells were used as target cells, a similar picture as with ARH-77 cells emerged (**FIG. 28**). Both 2F2T and 11B8T induced MNC-mediated lysis of Raji cells, albeit that 2F2T seemed more potent than 11B8T at low concentrations. The maximum lysis reached with 2F2T and 11B8T was approximately 35%. Rituximab induced MNC-mediated lysis of Raji cells, although only 20% of target cells were susceptible to rituximab. Addition of the isotype control antibody (HuMab-KLH) did not induce ADCC. No specific lysis was observed without addition of MNCs (data not shown).

Example 8

FRET and Triton-X Insolubility Analysis

[0461] Preparation of Cy3- and Cy5-conjugated mAb for fluorescence resonance energy transfer (FRET): Monoclonal antibodies were directly conjugated to bifunctional NHS-ester derivatives of Cy3 and Cy5 (Amersham Biosciences UK Ltd) as described in the manufacturer's instructions. Briefly, mAb were dialyzed against 0.1 M carbonate/bicarbonate buffer (pH 9). Thereafter, dye was dissolved in H_2O, immediately added to 1 mg of the mAb, and incubated at room temperature in the dark for 45 min. The labeled mAbs were separated from the unconjugated dye by gel chromatography using a PD10-Sephadex G25 column equilibrated in PBS. Molar ratios of coupling were determined spectrophotometrically from ϵ_{552}=150/mM/cm for Cy3, ϵ_{650}=250/mM/cm for Cy5, and ϵ_{628}=170/mM/cm for protein, and ranged from 5- to 8-fold excess dye:protein.

[0462] FRET analysis: Daudi cells were resuspended at 5×10^5 cells/ml in PBS/0.1% BSA, and equimolar donor (Cy3)-conjugated and acceptor (Cy5)-conjugated mAb were combined and added to the cell suspension (final concentration 10 μg/ml). Cells were incubated for 30 min in the dark, at 4° C. or 37° C. Each experiment included cells labeled with donor- and acceptor-conjugated mAb after pre-incubation with a 20-fold molar excess of unconjugated mAb, and cells labeled with donor- or acceptor-conjugated mAb in the presence of equimolar unlabeled mAb. To assess the association of labeled antigens, flow cytometric FRET measurement was carried out using a FACScalibur (BD Biosciences). The fluorescence intensities at 585 nm (FL2) and 650 nm (FL3), both excited at 488 nm, and the fluorescence intensities at 661 nm (FL4), excited at 635 nm, were detected and used to calculate FRET according to the equitation below, where A is acceptor (Cy5), and D is donor (Cy3). All values obtained were corrected for autofluorescence using the following formula:

[0463] FRET=FL3(D,A)-FL2(D,A)/a-FL4(D,A)/b

[0464] where a=FL2(D)/FL3(D), and b=FL4(A)/FL3(A)

[0465] Correction parameters were obtained using data collected from single-labeled cells, and side angle light scattering was used to gate out debris and dead cells. FRET between donor and acceptor mAb derivatives on dually labeled cells is expressed in terms of acceptor sensitized emission at 488 nm. Larger FRET values indicate closer physical association of the donor- and acceptor labeled antibodies or a higher density of acceptor-labeled mAb in the vicinity of donor-labeled mAb.

[0466] Assessment of raft associated antigen by Triton X-100 (TX) insolubility: As a rapid assessment of the presence of antigen in raft microdomains, a flow cytometry method based on Triton X-100 (TX) insolubility at low temperatures was used, as described previously In brief, Daudi cells were washed in RPMI/1% BSA and resuspended at 2.5×10^6/ml. The cells (100 μl) were then incubated with 10 μg/ml of FITC conjugated mAb for 15 min at 37° C., washed in cold PBS/1% BSA/20 mM sodium azide (PBS-BS), and the sample was divided in half. All samples were kept on ice throughout the remainder of the assay. One half was maintained on ice to allow calculation of 100% surface antigen levels, whilst the other was treated with 0.5% TX for 15 min on ice to determine to proportion of antigens remaining in the insoluble raft fraction. Cells were then maintained at 4° C. throughout the remainder of the assay, washed once in PBS-BS, resuspended in PBS-BS and assessed by flow cytometry. To determine the constitutive level of raft association of the target antigens, cells were first treated with 0.5% TX for 15 min on ice and washed in PBS-BS prior to binding of FITC-labeled mAb.

[0467] As shown in **FIGS. 29A, 29B**, and **29**, fluorescence resonance energy transfer (FRET) analysis indicates clustering of CD20 upon incubation with 2F2 or 7D8. No such clustering was observed upon incubation with 11B8. These results are consistent with the TX treatment data, cf. **FIG. 29C**, (i.e., 2F2 and 7D8, unlike 11B8 remain with the insoluble fraction of the cell following binding) and support the concept that 2F2 and 7D8, upon binding, translocate CD20 into lipid raft compartment of the B cell membrane.

[0468] As shown if **FIG. 30** (FRET values and s.e.m. of three experiments using one-way ANOVA followed by Tukey's multi comparison post-hoc test) the FRET analysis indicates clustering of rituximab and 2F2, whereas no clustering was observed with 11B8T. These data are in agreement with the data obtained after treatment with 0.5% TX prior to binding of FITC-labeled mAbs as shown in **FIG. 31** (n=2).

[0469] Preparation of lipid raft fractions and Western blotting: Another way to examine the association of CD20 with lipid rafts, is to investigate the distribution of CD20 between the raft and non-raft membrane fractions using the sucrose gradient fractionation method as disclosed by Deans, J. P., et al., *J. Biol. Chem.*, 1998. 273(1): pp 344-348, except that Optiprep (Sigma) was used instead of sucrose. Monoclonal antibodies directed against CD20 (10 μg/ml) were allowed to bind to Daudi cells (1×10^7) for 20 min at 37° C. Following this incubation, the cells were pelleted, washed twice with PBS and lysed in ice-cold lysis buffer (1.0% TX in MES-buffered saline (25 mM MES, pH 6.5, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 10 mM EDTA)). The cell pellet was resuspended thoroughly and incubated for 20 min on ice. Thereafter, the lysate was mixed with 400 μl cold 60% Optiprep (Sigma). The sample was overlaid with a 600 μl step of each 35%, 30%, 25%, 20%, 0% Optiprep in lysis buffer. The gradients were spun at 40.000 rpm at 4° C. for 18 hours. Six fractions from the top were collected, resolved on a 4-15% SDS-PAGE gel, transferred onto nitrocellulose membranes and incubated with primary antibody (mouse anti-CD20 polyclonal; Serotec, UK), followed by HRP-conjugated secondary antibody (rabbit anti mouse-HRP; Jackson, Bar Harbor, Me., USA). Blots were visualized using Supersignal West Dura extended duration substrate (Pierce, Woburn, Mass., USA).

[0470] The results are shown in **FIG. 32**. As it can be seen, CD20 molecules are confined to the high-density fraction 5 (untreated cells). Cells treated with rituximab showed a distinct shift in CD20 distribution with a significant proportion in the lower density membrane fractions 2 and 3, coincident with the fraction where membrane rafts are expected to sediment. Cells treated with 2F2 also showed this shift to fractions 2 and 3. In contrast, cells treated with 11B8T for 20 min showed a similar distribution to untreated cells, with CD20 molecules in fraction 5. In conclusion binding to 2F2 and rituximab induces a shift of CD20 molecules to the lower density membrane fractions, whereas binding to 11B8T does not.

Example 9

Apoptosis of Burkitt Cell Lines with Human Antibodies Against CD20

[0471] Apoptosis: Daudi cells, 0.5×10^6 in 1 ml tissue culture medium, were placed into 24-well flat-bottom plates with 1 or 10 μg/ml mAb or control antibodies, and incubated at 37° C. After 20 hours, cells were harvested, washed in Annexin-V-FITC binding buffer (BD biosciences) and labeled with Annexin V-FITC (BD biosciences) for 15 min in the dark at 4° C. The cells were kept at 4° C. until analysis. Each sample (150 μl) was added to 10 μl of PI solution (10 μg/ml in PBS) in a FACS tube. The mixture was assessed immediately by flow cytometry using a FACScalibur flow cytometer with CellQuest pro software (BD Biosciences, Mountain view, Calif.). At least 10,000 events were collected for analysis.

[0472] Induction of apoptosis in Daudi cells: Daudi cells were incubated for 20 hours in the presence of human antibodies against CD20 (1 μg/ml) (without the addition of a secondary cross-linking antibody). Induction of apoptosis was assessed by AnnexinV/PI staining using flow cytometry.

[0473] As shown in FIGS. **33A-G**, 11B8 shows clear evidence of inducing apoptosis (similar to that induced by an anti-IgM antibody). 2F2 and 7D8 did not induce apoptosis of Daudi cells. An apoptosis-inducing mouse anti-CD20 antibody, AT80, was used as a control.

[0474] Induction of apoptosis in Raji cells: Induction of apoptosis of Raji cells was tested with a concentration range of CD20 mAbs. **FIG. 34** shows the percentage of annexin-V-positive cells. As can be seen from **FIG. 34**, the positive control mouse anti-human CD20-mAb, B1, induced a concentration-dependent increase in apoptosis of Raji cells with a maximum of approximately 70% at 10 μg/ml mAb. Also 11B8 was a strong inducer of apoptosis, resulting in apoptosis of Raji cells with a maximum of 53.4% at 10 μg/ml mAb. On the other hand, 2F2 and rituximab were very poor in inducing apoptosis of Raji cells, with slightly elevated levels of apoptosis compared to negative control levels.

[0475] Induction of apoptosis in Daudi cells: The same picture emerged when Daudi cells were used as target cells, after addition of 1.0 μg/ml CD20 mAb (**FIGS. 35A and 35B**). Data in **FIG. 35A** show the total of annexin-V positive cells, and data in **FIG. 35B** (the X-axis showing annexin-V, and the Y-axis showing PI) show the percentages of Daudi cells in early apoptosis (annexin-V positive and PI negative) and late apoptosis (annexin-V positive and PI positive). Again, Both B1 (65.9%) and 11B8T (56.3%) were strong inducers of apoptosis (**FIG. 36**), when used at a concentration of 1.0 μg/ml. Addition of 2F2T resulted in a low level of apoptotic Daudi cells (17%). Addition of rituximab resulted in approximately 29% apoptosis of Daudi cells. Addition of isotype control antibody HuMab-KLH did not induce apoptosis of Daudi cells (6%).

Example 10

Homotypic Adhesion of Cells with Human Antibodies Against CD20

[0476] Homotypic aggregation correlates with induction of apoptosis. Therefore, the ability of the anti-CD20 mAbs to induce homotypic aggregation of B cells was investigated

[0477] Homotypic aggregation of Ramos-EHRB cells: Ramos-EHRB cells (0.5×10^6 in 1 ml tissue culture medium) were incubated at 37° C. for 4 hours in the presence of anti-CD20 antibodies 11B8, 2F2, or 7D8 (without cross-linking) and induction of homotypic adhesion was assessed by light microscopy (as described above).

[0478] As shown in FIGS. 36A-E, 11B8 caused extensive aggregation of Ramos-EHNB cells (similar to the aggregation caused by murine anti-CD20 antibody, AT80). 2F2, and 7D8 did not induce homotypic aggregation of Ramos cells.

[0479] Homotypic aggregation of Daudi cells: Daudi cells were placed into 24-well flat-bottom plates with 1 or 10 μg/ml anti-CD20 mAbs or control antibody, and incubated at 37° C. for 4 hours. The extent of homotypic aggregation was determined by light microscopy. As can be seen from **FIG. 37**, 2F2 hardly induced homotypic aggregation of Daudi cells, with 1.0 μg/ml (and 10 μg/ml, data not shown). Rituximab gave little homotypic aggregation of Daudi cells. In contrast, the B1 antibody was a strong inducer of homotypic aggregation.

Example 11

Immunotherapy Using Human Antibodies Against CD20

[0480] Therapy with high dose (100 μg) 2F2 and 7D8 of SCID mice challenged with Daudi cells: The SCID mice were obtained from Harlan UK Ltd., Blackthorn, Oxon, UK, and bred and maintained under pathogen free conditions. Daudi cells (2.5×10^6) were injected i.v. into the tail vein of cohorts of 12-16 weeks old SCID mice, followed 7 days later by injection of 100 μg of 2F2 or 7D8 via the same route. Animals were sacrificed upon presentation of limb paralysis, according to the instructions of the animal ethics committee. As shown in **FIG. 38**, survival of the mice is prolonged after treatment with 2F2 or 7D8.

[0481] Therapy with high dose (100 μg) 2F2 and rituximab of SCID mice challenged with Tanoue cells: Tanoue cells (2.5×10^6 in 200 μl PBS) were injected i.v. into the tail vein of cohorts of 12-16 week old SCID mice (Harlan UK Ltd., Blackthorn, Oxon, UK) followed 7 days later by the injection of 100 μg (in 200 μl PBS) of anti-CD20 mAb via the same route. In this experiment, 2F2 was compared to rituximab and B1. Animals were sacrified upon presentation of rear-limb paralysis. The results are shown in **FIG. 39**. At day 39, the first two control mice died, and death within this group was complete at day 54. Only one mouse died within this time interval following 2F2 treatment and survival was considerably increased for the other mice in this group. One mouse died 81 days following injection of the tumor cells and the remaining mice (60% of the total number) survived beyond the termination of the experiment at 100 days post tumor challenge. Rituximab in contrast only increased survival for 2 out of 5 mice (dying at 66 and 83 days post challenge) and none of the mice survived until the end of the experiment. In the B1-group, the survival of SCID mice was similar to that in the 2F2 group, with two mice dying on day 48, and one mouse on day 76. In this group, forty percent was alive at the time the experiment was terminated.

[0482] Dose response of 2F2 and rituximab treatment of SCID mice challenged with Daudi cells: To assess the efficacy of 2F2 in comparison to rituximab in protection against tumorigenesis, a dose titration was performed in therapy of SCID mice challenged with Daudi tumor cells. Daudi cells express more CD20 than Tanoue cells and are more sensitive to killing in vitro. 10 groups of SCID mice (4 per group) and 1 control group (5 SCID mice) were injected with 2.5×10^6 Daudi cells (in 200 μl PBS) i.v. on day 0, and then treated with 20, 5, 2, 0.5 or 0.1 μg (in 200 μl PBS) rituximab, 2F2 or PBS (control) i.v. on day 7. Animals were sacrificed upon presentation of rear-limb paralysis. The results are shown in **FIG. 40**.

[0483] In the control group, all mice died within the time interval of 26-29 days. However, a clear dose-effect relation was observed with 2F2 (**FIG. 40**, upper graph). Whereas no effect was observed with doses of 0.1 μg and 0.5 μg 2F2, as little as 2 μg 2F2 substantially extended survival until day 41, 5 μg 2F2 extended survival until day 47, and 20 μg 2F2 extended survival even until day 50.

[0484] In contrast, rituximab even tested at the highest dose of 20 μg only slightly increased survival and no dose-effect relation was therefore observed at the lower concentrations tested (**FIG. 40**, lower graph).

[0485] Therapy of SCID mice with Daudi tumors by 11B8T and B1: Daudi cells (2.5×10^6) in 200 μl PBS were injected i.v. into the tail vein of cohorts of 12-16 week old SCID mice, followed 7 days later by the injection of 100 μg 11B8 or B1 in 200 μl PBS via the same route. Animals were sacrificed upon presentation of rear-limb paralysis. In control mice treated with PBS, all mice died within a time interval of 35-53 days (**FIG. 41**). 11B8T treatment strongly protected the mice, with mice dying between 72 and 98 days post tumor challenge. In the B1-treatment group, most mice survived until day 98 and 40% of the mice survived beyond the end of the experiment, i.e., day 100.

Example 12

Evaluation of Anti-CD20 Antibodies in a Daudi-luc Xenograft Model Using SCID Mice

[0486] The therapeutic efficacy of anti-CD20 antibodies was evaluated in a mouse model in which disseminated outgrowth of human B-cell tumor cells is followed using external optical imaging. In this model tumor cells are transfected with firefly luciferase. Upon administration of luciferin (Molecular Probes, Leiden, The Netherlands) to the mice the labeled cells can be detected in vivo by bioluminescent imaging using a highly sensitive CCD camera, cf. Wetterwald et al. (2002) *American Journal of Pathology*, 160(3):1143-1153.

[0487] Daudi cells were transfected with gWIZ luciferase from Gene Therapy Systems (San Diego, Calif.) and cultured in RPMI with 10% FCS, Pen/Strep, Sodium Pyruvate and 1 μg/ml puromycin (Sigma). Cells were analysed for luciferase expression (expressed in RLU/1×10^5 cells) in a luminometer and for CD20 expression by FACS. 2.5×10^6 luciferase-transfected Daudi cells/mouse were injected i.v. into SCID mice. Eight days after inoculation, the mice received a single dose (10 μg) treatment of 2F2T, 11B8T, rituximab, B1 or isotype control antibody (huIgG1) (6 mice per treatment group). For imaging, mice were anesthetized by i.p. injection of a mixture of ketamine/xylazine/atropine. Synthetic D-Luciferin (sodium salt, Molecular Probes) was given i.p. at a dose of 25 mg/ml. Mice were then placed in a light tight box and after 3 min, imaging was started using a VersArray 1300F liquid nitrogen cooled CCD detector (Roper Scientific). Photons emitted from the luciferase were counted over an exposure period of 5 min. Under illumination black and white images were made for reference. MetaVue software (Universal Imaging Corp) was used for

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DIRECCIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. PCT 10-23468

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCIÓN**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **626** DE

FECHA **22 DE MARZO DE 2011** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **17 DE JUNIO DE 2011**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI ____X_____ NO _____

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Bogotá, D.C.,

Doctora:
Luz Clemencia De Páez.
Apoderada

Oficio N°: 010884

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Actuación	440
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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 Eliana Isaura Moreno Bohórquez, 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.

CARLOS DANIEL LEMUS GIRALDO.
Profesional Universitario 2044-09 (P).
Con asignación de funciones de la
Dirección de Nuevas Creaciones.

El anterior oficio fue notificado por fijación en lista de fecha, 14 JUL. 2011.

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