

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

2020
Bogotá, D.C.,

Abogada
LUZ CLEMENCIA DE PÁEZ
cavelier@cavelier.com

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 09-081882- -00005-0000

Fecha: 2012-09-14 09:16:09 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 519 TRASLADOINFORME Folios: 7

ASUNTO	Radicación	09-081882
	Trámite	11
	Evento	378
	Actuación	519
	Oficio	
	Folios	7

16502

Patente de Invención ☒

Patente de Modelo de Utilidad ☐

Vía Nacional ☐

Vía PCT (Fase Nacional) ☒

Capítulo I ☒

Capítulo II ☐

No. Solicitud Internacional PCT/EP2008/000053

Fecha de Presentación Internacional 07/Enero/2008

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 171 a 359	5/Agosto/2009
Reivindicaciones:	Folios 360 a 369	5/Agosto/2009
Dibujos:	Folios 371 a 380	5/Agosto/2009
Listado de Secuencias:	Folios 381 a 406	5/Agosto/2009
Prioridad:	US 60/878,831	5/Enero/2007
	EP 07000211.8	5/Enero/2007
	US 60/934,291	11/Junio/2007
	EP 07020341.9	17/Octubre/2007

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486), y su contenido técnico corresponde con el de la prioridad reivindicada.

Expediente 09-081882 1er Art 45

3. Objeto de la invención

Título de la solicitud: "Un método para aislar una molécula de enlace específico derivado de desórdenes asociados a proteínas, moléculas de enlace específicas y objetivos" (modificado en el folio 421).

El problema planteado en esta solicitud consiste en la necesidad de suministrar agentes que sean específicos para un objetivo implicado en un trastorno y que sean tolerados por el cuerpo humano.

Para resolver este problema técnico la solicitud en estudio proporciona un método para aislar una molécula de enlace específica.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07K 016/018.

4. No es una invención (Art 15 D 486 literal (b))

El objeto de las reivindicaciones 1 – 11 no se considera una invención porque hace referencia a un proceso biológico natural, que es la producción de anticuerpos por una célula presente en una muestra de un sujeto humano al entrar en contacto ésta con un antígeno.

El objeto de las reivindicaciones 14 – 23, 26 – 29 y 34 no se considera una invención, de acuerdo con el Art citado, porque hace referencia a material biológico existente en la naturaleza o aquel que puede ser aislado.

5. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 43 – 46 no es patentable de acuerdo con el Art citado.

6. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 41, 42 y 48 no es patentable, de acuerdo con el Art 14.

7. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1, 2, 4, 12, 16, 24, 25, 26 28 – 30, 34 – 36 y 49 – 51 carecen de concisión porque utilizan las expresiones "una muestra obtenida de un paciente", "un fluido corporal", "un anticuerpo", "identificar y opcionalmente aislar", "purificar células B o células de memoria B, "que comprende en su región variable al menos una región determinante de complementariedad (CDR) como se describe en la tabla 4", "una secuencia de aminoácido de la región VH y/o VL como se describe en las Tablas 2 y 3", "un antígeno", "un polinucleótido", un

vector", "etiquetada de una manera detectable", "una enzima", "un radioisótopo", "un fluoróforo", "un metal pesado", "un fármaco", "NI-101.10 (...)" lo cual dificulta determinar la extensión del objeto que se busca proteger.

Para las expresiones "que comprende en su región variable al menos una región determinante de complementariedad (CDR) como se describe en la tabla 4", "una secuencia de aminoácido de la región VH y/o VL como se describe en las Tablas 2 y 3", "un polinucleótido", un vector", "NI-101.10, NI-101.11, NI-101.12, NI-101.13, NI-101.12F6A, NI-101.13A, y NI-101.13b", se recuerda al solicitante que un polipéptido y un ácido nucleico se caracterizan por sus secuencias de aminoácidos y polinucleótidos, respectivamente. Así mismo, un anticuerpo se caracteriza por la secuencia de aminoácidos de las regiones variables de las cadenas pesada y ligera o la secuencia de aminoácidos de los 6 CDR. Se requiere al solicitante hacer referencia a un listado de secuencias.

Para las expresiones "identificar y opcionalmente aislar", "purificar células B o células de memoria B", se sugiere al solicitante definir las condiciones en las que se llevan a cabo estas etapas del método.

Las reivindicaciones 1, 14 – 22 y 26 utilizan las expresiones "que enlaza al espécimen pero no a las correspondientes células o tejidos de un sujeto saludable", "caracterizada porque es capaz de reconocer selectivamente un neopítipo de una proteína asociada con el trastorno", "porque es capaz de restablecer substancialmente el comportamiento normal", "caracterizado porque es reconocido por la molécula de enlace (...), que son resultados a alcanzar y no definen a la molécula puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se sugiere reemplazarlo por las características esenciales o estructurales que contribuyen a la obtención de ese efecto, como la secuencia de aminoácidos de las regiones variables de las cadenas pesada y ligera o la secuencia de aminoácidos de los 6 CDR.

Las reivindicaciones 37 – 40 reclaman una composición que comprende la molécula de enlace, el anticuerpo o fragmento de enlace, el antígeno, el polinucleótido, el vector o la célula. Se recuerda al solicitante que una composición se caracteriza por sus componentes y sus proporciones dentro de la misma.

La reivindicación 39 no es concisa porque reclama combinaciones de los agentes de la reivindicación 39 con un agente adicional útil para tratar la enfermedad de Alzheimer, seleccionado de moléculas orgánicas pequeñas. Anticuerpos Anti-beta y combinaciones, lo cual dificulta determinar la extensión del objeto que se busca proteger. Por demás se recuerda al solicitante que esta manera de reclamar no es apropiada, porque el segundo componente se está definiendo por su función o uso, lo cual no define inequívocamente un ingrediente activo como tal. Además en la descripción si bien se nombran, no se evidencia un efecto técnico generado por la combinación reclamada en la presente solicitud tal como sinergia, disminución de dosis, disminución de efectos secundarios etc. Por lo tanto se sugiere al

solicitante eliminar esta reivindicación del alcance del pliego reivindicatorio porque no encuentra soporte en la descripción.

Se requiere al Solicitante hacer las aclaraciones necesarias.

8. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: Enero 5 de 2007, y se encontró el siguiente documento que anticipa el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	<u>WO2006103116</u>	Human antibodies with beta-amyloid peptide-binding capacity and their applications	5/Octubre/2006

D1http://worldwide.espacenet.com/publicationDetails/biblio?CC=WO&NR=2006103116&KC=&FT=E&locale=en_EP divulga un método para aislar un anticuerpo que se une a la proteína amiloide beta (Pág. 5, párrafo 16 y Pág. 88, párrafos 374-376).

9. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La **reivindicación 12** consiste en el método de conformidad con una de cualquiera de las reivindicaciones 1 a 11, caracterizado además porque comprende las etapas de:

- (i) Purificar células B o células de memoria B de una muestra que se ha identificado que contiene moléculas enlazantes, por ejemplo anticuerpos que enlazan al espécimen pero no a las correspondientes células o tejido de un sujeto saludable;
- (ii) Obtener el repertorio de gen de inmunoglobulina para los anticuerpos a partir de las células B o células de memoria B; y
- (iii) Usar el repertorio para expresar los anticuerpos.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1
El método de conformidad con una de cualquiera de las reivindicaciones 1 a 11, caracterizado además porque comprende las etapas de: (i) purificar células B o células de memoria B de una muestra que se ha identificado que contiene moléculas enlazantes, por ejemplo anticuerpos que enlazan al espécimen pero no a las correspondientes células o tejido de un sujeto saludable; (ii) obtener el repertorio de gen de inmunoglobulina para los anticuerpos a partir de las células B o células de memoria B; y	Un método para aislar un anticuerpo que se une a la proteína amiloide beta que comprende someter una muestra obtenida de un paciente de una edad entre 60 – 100 años e identificar anticuerpos de unión a la proteína amiloide beta, caracterizado además porque comprende las etapas de: i) purificar células B; ii) obtener el repertorio de gen de inmunoglobulina para los anticuerpos a partir de las células B o células de memoria B; y iii) usar el repertorio para expresar los anticuerpos.

(iii)	usar el repertorio para expresar los anticuerpos.	(Pág. 5, párrafo 16 y Pág. 88, párrafos 374-376)
-------	---	--

Las características **esenciales** del método de la reivindicación 12 habían sido divulgadas previamente en D1, el cual enseña un método para aislar un anticuerpo que se une a la proteína amiloide beta que comprende someter una muestra obtenida de un paciente de una edad entre 60 – 100 años e identificar anticuerpos de unión a la proteína amiloide beta, caracterizado además porque comprende las etapas de: i) purificar células B; ii) obtener el repertorio de gen de inmunoglobulina para los anticuerpos a partir de las células B o células de memoria B; y iii) usar el repertorio para expresar los anticuerpos (Pág. 5, párrafo 16 y Pág. 88, párrafos 374-376). D1 también enseña que la obtención del repertorio de gen de inmunoglobulina comprende sintetizar el cADN del mRNA de las células B (Pág. 88, párrafo 376).

En consecuencia, la reivindicación 12 carece de novedad, según lo divulgado en el documento D1.

La reivindicación dependiente 13 no menciona alguna característica que haga nueva a la molécula de la reivindicación 12, por lo cual se considera que es nueva.

Nivel Inventivo (Art18 D486)

La reivindicación 24 consiste en la molécula de enlace de conformidad con una de cualquiera de las reivindicaciones 14 a 23, caracterizada porque es un anticuerpo o fragmento enlazante del mismo que comprende en su región variable al menos una región determinante de complementariedad (CDR) como se describe en la tabla 4.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1
La molécula de enlace de conformidad con una de cualquiera de las reivindicaciones 14 a 23, caracterizada porque es un anticuerpo o fragmento enlazante del mismo que comprende en su región variable al menos una región determinante de complementariedad (CDR) como se describe en la tabla 4.	Un anticuerpo que se une a la proteína amiloide beta que comprende las secuencias de aminoácidos SEQ ID NO: 2 y 4 (Pág. 9, párrafo 32)

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 24 porque divulga anticuerpo que se une a la proteína amiloide beta que comprende las secuencias de aminoácidos SEQ ID NO: 2 y 4 (Pág. 9, párrafo 32). D1 también enseña el ácido nucleico que codifica el anticuerpo, el vector que comprende el ácido nucleico, una célula hospedera que comprende el vector (Pág. 100, reivindicaciones 14-18), una composición farmacéutica y un kit que comprende el anticuerpo o el ácido nucleico para el tratamiento de la enfermedad de Alzheimer (Pág. 101, reivindicación 23, Pág. 104, reivindicación 42).

La diferencia entre la invención, definida en la reivindicación 24 y el anticuerpo de D1 consiste en que la solicitud reclama CDRs con secuencias específicas.

Esta diferencia no parece conceder un efecto técnico inesperado o una ventaja al anticuerpo reclamado en esta solicitud porque no parece presentar una mayor afinidad a las estructuras patológicas relacionadas con la enfermedad de Alzheimer.

El problema técnico objetivo de esta solicitud consiste en la necesidad de anticuerpos para el tratamiento de la enfermedad de Alzheimer alternativos a los ya conocidos en D1.

Teniendo en cuenta que D1 ya divulgaba un método para aislar un anticuerpo que se une a la proteína amiloide beta que comprende someter una muestra obtenida de un paciente de una edad entre 60 – 100 años e identificar anticuerpos de unión a la proteína amiloide beta, caracterizado además porque comprende las etapas de: i) purificar células B; ii) obtener el repertorio de gen de inmunoglobulina para los anticuerpos a partir de las células B o células de memoria B; y iii) usar el repertorio para expresar los anticuerpos (Pág. 5, párrafo 16 y Pág. 88, párrafos 374-376), y que el anticuerpo que se une a la proteína amiloide beta comprende las secuencias de aminoácidos SEQ ID NO: 2 y 4 (Pág. 9, párrafo 32), la persona del oficio medianamente versada en la materia habría inferido sin mucho esfuerzo de su parte, utilizar tejido cerebral para el tamizaje de anticuerpos que se unen a moléculas conocidas y relacionadas con la enfermedad de Alzheimer¹, para obtener más anticuerpos con la misma actividad.

De manera que la solución propuesta por esta solicitud en la reivindicación 24 y su dependiente 25 y en las reivindicaciones 30 – 33, 35 – 40, 47 y 49 – 51, es obvia y no tiene Nivel Inventivo.

10. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	<u>WO2006103116</u>	12, 13, 24, 25, 30 – 33, 35 – 40, 47 y 49 – 51	Novedad y Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

1 V. Geylis et al. Immunotherapy of Alzheimer's disease (AD): From murine models to anti-amyloid beta (Aβ) human monoclonal antibodies. Autoimmunity Reviews, Vol. 5, Pág. 33-39. Publicado en enero de 2006.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante para dar respuesta a este requerimiento y presentarla por escrito.

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 No. 10 de 2001.


JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha
18 SET. 2012

Elaborado por: Juan David Moncaleano P. 
Revisado por: Consuelo Leguizamón L. 



Immunotherapy of Alzheimer's disease (AD): From murine models to anti-amyloid beta (A β) human monoclonal antibodies[☆]

Valeria Geylis, Michael Steinitz*

Department of Pathology, The Hebrew University - Hadassah Medical School, Jerusalem, Israel

Received 25 May 2005; accepted 15 June 2005

Available online 1 August 2005

Abstract

The deposition of amyloid beta (A β) protein is a key pathological feature in Alzheimer's disease (AD). In murine models of AD, both active and passive immunization against A β induce a marked reduction in amyloid brain burden and an improvement in cognitive functions. Preliminary results of a prematurely terminated clinical trial where AD patients were actively vaccinated with aggregated A β bear resemblance to those documented in murine models. Passive immunization of AD patients with anti-A β antibodies, in particular human antibodies, is a strategy that provides a more cautious management and control of any undesired side effects. Sera of all healthy adults contain anti-A β IgG autoimmune antibodies. Hence antigen-committed human B-cells are easily immortalized by Epstein–Barr virus (EBV) into anti-A β secreting cell lines. Two anti-A β human monoclonal antibodies which we recently prepared bind to the N-terminus of A β peptide and were shown to stain amyloid plaques in non-fixed brain sections from an AD patient.

It is anticipated that specifically selected anti-A β human monoclonal antibodies could reduce and inhibit deposits of amyloid in brain while avoiding the cognitive decline that characterizes AD. In the future, this type of antibody may prove to be a promising immune therapy for the disease.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Alzheimer's disease; Amyloid beta; Epstein–Barr virus; Human monoclonal antibodies; Immunotherapy

Contents

1. Introduction	34
2. Studies in transgenic mouse models of AD.	34
3. Human studies	34

[☆] Grant support: This study was supported by grant awarded to V.G. by Abish-Frenkel Foundation.

* Corresponding author. Tel.: +972 2 6758204; fax: +972 2 6784010.

E-mail address: michaels@md.huji.ac.il (M. Steinitz).

3.1.	Anti-A β antibodies in human sera	34
3.2.	Active immunization	35
3.3.	Passive immunization	35
4.	Possible mechanisms of A β -clearance by anti-A β antibodies	35
5.	Active versus passive anti-A β immunization	36
6.	Human anti-A β monoclonal antibodies	36
7.	Conclusions	37
	Take-home messages	37
	References	37

1. Introduction

Alzheimer's disease (AD) is an incurable, disabling and devastating illness which affects a significant proportion of the elderly people in the Western world. Brain region-specific amyloid-beta (A β) deposits are a key pathological feature of the disease. Familial forms of AD have been linked to mutations in the amyloid precursor protein (APP) and the Presenilin genes. The current treatments of AD provide only symptomatic relief and are practically inefficient. Based on recent studies in murine models of AD, a novel approach for immune therapy of AD patients has been put forward. Accordingly, circulating anti-A β antibodies prevent de novo development and reduce existing deposits of harmful A β in the brain with a concurrent improvement of cognitive functions.

2. Studies in transgenic mouse models of AD

Several models of transgenic mice which express mutant forms of human amyloid precursor protein (APP) and Presenilin 1 (PS1) [1] exhibit an age-related AD-like phenotype, characterized by progressive cognitive impairment and accompanied by the accumulation of A β plaques in the brain. Immunization of young transgenic mice with a synthetic A β peptide inhibited amyloid deposition in the brain, whereas in older mice, already exhibiting the characteristic amyloid plaques, there was a marked reduction in brain amyloid burden [2,3]. Moreover, there was an improvement in the cognitive level following vaccination of mice with the A β peptide [4–7]. These effects were documented also in transgenic mice that were passively immunized with rabbit polyclonal or murine monoclonal anti-A β antibodies [8–11]. Anti-A β antibodies were shown to

penetrate into the brain of passively immunized mice [12] and “decorate” the amyloid plaques [8]. This is convincing evidence that in the mice with an intact brain–blood barrier (BBB), the anti-A β antibodies were accessible to the brain. Interestingly, some of the injected monoclonal anti-A β antibodies did not affect the amyloid burden in vivo despite the fact that they bound the A β peptide in vitro [8]. Thus, the specific binding of synthetic A β peptide is not by itself a proof of the in vivo effectiveness of the antibody.

3. Human studies

3.1. Anti-A β antibodies in human sera

APP is a normal constituent of the membrane in different types of cells and A β is one of its breakdown products. Several studies showed naturally occurring autoimmune anti-A β antibodies in sera of all healthy adults and also in AD patients [13–18]. The fact that some studies claim higher titres in AD patients whereas others show the contrary is probably related to the differences in the form of the A β antigen used in the anti-A β ELISA's. Significantly, quantitative assessment of anti-A β antibodies by ELISA using an antigen that has a multifaceted structure is most likely misleading. However, qualitative differences of anti-A β antibodies such as affinity, epitope specificity and biological characteristics probably would be unveiled by using more sophisticated assays. Thus, anti-A β antibodies derived from healthy adults may induce completely different effects in vivo if compared to equal concentrations of anti-A β antibodies from AD patients. The diversity of anti-A β antibodies in normal human serum, in relation for example to epitope specificity, is mirrored in intrave-

nous immunoglobulins (IVIG), a commercially available preparation of pooled human immunoglobulins [18]. In fact, IVIG is an excellent source of physiologically normal non-pathogenic anti-A β antibodies [19–21]. Molecular analysis of the v-region of anti-A β antibodies in normal human serum, the relatively high serum titres of these antibodies and the fact that serum anti-A β antibodies are IgG's [15–18] support the notion that the antibodies are induced by a specific antigen-driven immune process [22] and that they probably fulfill a physiological function in the homeostasis of APP and A β .

3.2. Active immunization

The promising results of immune therapy in the murine AD models focused increased interest on a similar approach in patients. A multicenter clinical trial was initiated whereby AD patients were immunized with pre-aggregated A β peptide (AN1792). The trial was terminated prematurely because some patients developed symptoms consistent with meningoencephalitis [23–28]. Although the results of the incomplete clinical trial are consistent with the murine model they cannot unequivocally confirm that active A β vaccination induces a dramatic improvement in the pathological symptoms. Brain tissue of the actively immunized patients had an atypically low number of diffuse and neuritic plaques but showed no effect in regard to the vascular amyloid. The collapsed brain plaques in the treated patients were surrounded by activated microglia and multinuclear giant cells filled with an extremely dense amyloid [25,27]. The majority, but not all of the vaccinated patients, developed anti-A β antibodies that recognized brain β -amyloid plaques [23,24,26,28]. There was an inverse correlation between the increase in serum anti-A β antibodies and the progression of the disease. Patients with brain-reactive anti-A β antibodies had a significant slower rate of cognitive decline than patients without brain-reactive antibodies [24]. In some patients the induced anti-A β antibodies crossed the BBB and were detected in CSF [23,24,26]. The meningoencephalitis that occurred in some of the patients was unrelated to their induced anti-A β antibody titre [24] but rather referred to involvement of specific T lymphocytes [25,27].

3.3. Passive immunization

IVIG antibodies were shown to interfere in vitro with the fibrillation of A β and to protect neurons exposed to toxic concentrations of A β peptide [20]. In a clinical trial, passive immunization of AD patients with anti-A β antibodies purified from IVIG markedly reduced the concentration of A β peptide in the CSF and similarly increased serum A β , suggesting an antibody-induced exit of A β from the brain to the periphery. Worth mentioning is that these passively immunized patients exhibited a certain improvement in cognitive functions without hazardous side effects [21].

4. Possible mechanisms of A β -clearance by anti-A β antibodies

It is generally accepted that A β peptide is the incriminating molecule that is responsible for the neural damage in AD. This notion is further supported by the close association between the intensified presence of amyloid plaques in the brain and the concurrent cognitive impairment in AD patients. The fact that in murine models of AD a reversal of cognitive impairment that follows active A β immunization is not necessarily accompanied by a visible change in A β burden in the brain suggests that even non-deposited forms of A β play a role in the pathogenesis of the disease [6,7].

The mechanism(s) by which anti-A β antibodies affect the clearance of A β from the brain is not yet clear. Three hypotheses have been proposed:

- 1) Fc-mediated phagocytosis. Accordingly, the clearance of A β is accomplished by microglia cells attracted to the plaques that are "decorated" with anti-A β antibodies [8,9]. In an ex vivo assay with brain tissue of transgenic mice and AD patients, only anti-A β antibodies which were "protective" in vivo triggered microglial Fc-receptor-mediated phagocytosis and subsequent A β peptide degradation [8]. The finding that in a murine model of AD, F(ab)₂ fragments of anti-A β antibodies were as effective in clearance of A β as the full-length antibody indicated that Fc-dependent phagocytosis was not the only mechanism involved [29].

- 2) "Peripheral sink" mechanism. Clearance of amyloid from the brain is accomplished by sequestration of A β from the CNS to the peripheral blood in the presence of anti-A β antibodies in the plasma [6,11,19,21,30]. This theory is not compatible with the finding that in active A β immunization of AD patients the levels of A β in both CSF and plasma are unaffected [24].
- 3) Inhibition of fibrillar aggregation. According to this hypothesis anti-A β antibodies directed specifically against A β epitopes involved in the fibrillization process antagonize the deposition of amyloid [31,32].

Antibody-induced inhibition of amyloid deposits and clearance of preexisting ones is probably accomplished by more than one mechanism. It has been suggested [33] that a two-phase mechanism of clearance is involved in the brain. First, diffuse A β molecules are rapidly cleared without microglial activation and second, a lengthy clearance of compact A β follows, involving activation of microglial cells and Fc-mediated phagocytosis of pre-existing plaques.

In murine models of AD, effective clearance of A β plaques *in vivo* was accomplished by anti-A β antibodies that protected *in vitro* against A β -induced neural injury; stained A β plaques in brain sections and bound *in vitro* to an N-terminal epitope of A β . In contrast, the ability of antibodies to bind soluble A β did not necessarily correlate with the efficacy of the antibodies to clear amyloid plaques *in vivo* [8,9,32]. In A β -immunized AD patients, improvement of cognitive functions was found to be associated with the intensity of *in vitro* staining of A β plaques but not with patients' anti-A β antibody titres [24].

5. Active versus passive anti-A β immunization

Both active and passive strategies of anti-A β immunization are effective in the mouse models of AD. Active vaccination in AD patients induced meningoencephalitis in some of the patients. There was no apparent correlation between meningoencephalitis incidence among the treated patients and the magnitude of the induced anti-A β antibody titres [23,24]. This adverse outcome of the active immunization referred primarily to T-cell response [25,27]. It

is assumed that in contrast to active immunization, passively administered antibodies would not elicit such a hazardous cellular response and therefore passive anti-A β immunization is today more acceptable than the active vaccination. Remarkably, anti-A β antibody response in A β -immunized AD patients was unpredictable and some cases even failed altogether to develop anti-A β antibodies [23]. A regulation and continuous monitoring of the dose of infused antibody and the frequency of administration allow a reliable handling of any adverse side effects that might follow the passive immune therapy. In addition, detrimental effects that may be induced by passive immunotherapy would subside within a short time while similar effects that follow active immunization would probably be long-lasting.

6. Human anti-A β monoclonal antibodies

Antigen-committed B cells can be immortalized *in vitro* by Epstein-Barr virus (EBV) to produce human IgM, IgG and IgA monoclonal antibodies [34–37]. Earlier, anti-A β antibodies were detected very infrequently in cell culture supernatants derived from EBV-transformed lymphocytes from AD patients and healthy individuals [38]. Recently we showed that cell lines that produce human monoclonal anti-A β antibodies can be established from nearly every healthy adult [18]. In practice, A β -committed B cells are isolated using A β coated magnetic beads, immortalized with EBV and finally the cells are cloned. The selection of A β -committed B cells is a prerequisite because of the low frequency of the specific cells in peripheral blood lymphocytes (PBL). We analyzed two IgM antibodies against epitope(s) located within amino acids 1–16 at the N-terminus of the A β peptide. This N-terminal region encloses the site involved in fibrillation of A β . The two antibodies bind A β only and do not bind other amyloids, thus the antibodies do not recognize epitope(s) which characterize the common cross β -pleated sheet structure. One of the antibodies stained the immature diffuse and core amyloid plaques in frozen sections of AD brain. Thus, the binding to a seemingly fibrillation epitope and the binding to native, non-fixed A β -plaques make this antibody a possible prototype model for an AD-protective anti-A β human monoclonal antibody.

In PBL, the majority of the B-cells express IgM whereas memory CD27⁺, IgG⁺ cells comprise only 15% of B-lymphocytes [39]. Hence, IgM cell lines are generally the most frequent outcome of EBV-infected PBL. A dramatic increase in the efficiency of EBV-induced immortalization of PBL including IgG⁺, CD27⁺ memory B-cells is attained if EBV is used in combination with a CpG deoxyoligomer [40]. CpG activates memory B cells to proliferate and differentiate making them susceptible to the immortalizing effect of EBV. Recently we used CpG in combination with EBV to produce IgG antibodies directed against the N-terminal region of A β [Geylis, to be published].

7. Conclusions

Active and passive anti-A β immunotherapies have been shown to reduce A β burden and improve cognitive functions in both murine models of AD and in AD patients. A clinical trial with AD patients immunized with pre-aggregated A β was prematurely stopped because of unforeseen serious side effects attracting increased interest in anti-A β passive immunization. It is tempting to speculate that human monoclonal antibodies corresponding to in vivo non-pathogenic antibodies could be useful for passive immunotherapy of AD. They are readily prepared by immortalization of autoimmune A β -committed cells from nearly every healthy adult. The monoclonal antibodies, which most likely resemble those involved normally in the homeostasis of APP and A β , would be tolerated when administered to patients and probably would be devoid of any hazardous side effect.

Take-home messages

- In murine models of AD, both active and passive immunization against A β induce a marked reduction in amyloid brain burden and an improvement in the cognitive functions.
- Preliminary results from a clinical trial where A β was used for active immunization of AD patients are in accord with those of the murine model.
- Serious adverse side effects that followed the active anti-A β vaccination of the AD patients suggested alternative passive immunotherapy. Passive immu-

nization with anti-A β antibodies is considered to be a more cautious strategy and is expected to be more efficiently controlled and better managed.

- In sera of all healthy adults the presence of a variety of autoimmune anti-A β IgG antibodies hints at the possibility of their normal physiological role.
- Human monoclonal anti-A β secreting cell lines can be established from nearly every healthy adult by immortalization of specific B cells with Epstein-Barr virus (EBV).
- In vitro produced human monoclonal anti-A β antibodies which resemble physiologically AD-protective antibodies would therefore be attractive candidates for future immunotherapy of AD.

References

- [1] Younkin SG. Amyloid beta vaccination: reduced plaques and improved cognition. *Nat Med* 2001;7:18–9.
- [2] Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 1999;400:173–7.
- [3] Sigurdsson EM, Scholtzova H, Mehta PD, Frangione B, Wisniewski T. Immunization with a nontoxic/nonfibrillar amyloid-beta homologous peptide reduces Alzheimer's disease-associated pathology in transgenic mice. *Am J Pathol* 2001;159:439–47.
- [4] Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 2000;408:979–82.
- [5] Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 2000;408:982–5.
- [6] Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, et al. Immunization reverses memory deficits without reducing brain A β burden in Alzheimer's disease model. *Nat Neurosci* 2002;5:452–7.
- [7] Kotilinek LA, Bacskai B, Westerman M, Kowarabayashi T, Younkin L, Hyman BT, et al. Reversible memory loss in a mouse transgenic model of Alzheimer's disease. *J Neurosci* 2002;22:6331–5.
- [8] Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, et al. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med* 2000;6:916–9.
- [9] Bard F, Barbour R, Cannon C, Carretto R, Fox M, Games D, et al. Epitope and isotype specificities of antibodies to beta-

- amyloid peptide for protection against Alzheimer's disease-like neuropathology. *Proc Natl Acad Sci U S A* 2003;100:2023–8.
- [10] Bacskai BJ, Kajdasz ST, Christie RH, Carter C, Games D, Seubert P, et al. Imaging of amyloid-beta deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. *Nat Med* 2001;7:369–72.
 - [11] DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 2001;98:8850–5.
 - [12] Banks WA, Terrell B, Farr SA, Robinson SM, Nonaka N, Morley JE. Passage of amyloid beta protein antibody across the blood-brain barrier in a mouse model of Alzheimer's disease. *Peptides* 2002;23:2223–6.
 - [13] Du Y, Dodel R, Hampel H, Buerger K, Lin S, Eastwood B, et al. Reduced levels of amyloid beta-peptide antibody in Alzheimer's disease. *Neurology* 2001;57:801–5.
 - [14] Hyman BT, Smith C, Buldyrev I, Whelan C, Brown H, Tang MX, et al. Autoantibodies to amyloid-beta and Alzheimer's disease. *Ann Neurol* 2001;49:808–10.
 - [15] Weksler ME, Relkin N, Turkenich R, LaRusse S, Zhou L, Szabo P. Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. *Exp Gerontol* 2002;37:943–8.
 - [16] Nath A, Hall E, Tuzova M, Dobbs M, Jons M, Anderson C, et al. Autoantibodies to amyloid beta-peptide (A beta) are increased in Alzheimer's disease patients and A beta antibodies can enhance A beta neurotoxicity: implications for disease pathogenesis and vaccine development. *Neuromolecular Med* 2003;3:29–39.
 - [17] Mruthini S, Buccafusco JJ, Hill WD, Waller JL, Jackson TW, Zamrini EY, et al. Autoimmunity in Alzheimer's disease: increased levels of circulating IgGs binding A beta and RAGE peptides. *Neurobiol Aging* 2004;25:1023–32.
 - [18] Geylis V, Kourilov V, Meiner Z, Nennesmo I, Bogdanovic N, Steinitz M. Human monoclonal antibodies against amyloid-beta from healthy adults. *Neurobiol Aging* 2005;26:597–606.
 - [19] Dodel R, Hampel H, Depboylu C, Lin S, Gao F, Schock S, et al. Human antibodies against amyloid beta peptide: a potential treatment for Alzheimer's disease. *Ann Neurol* 2002;52:253–6.
 - [20] Du Y, Wei X, Dodel R, Sommer N, Hampel H, Gao F, et al. Human anti-beta-amyloid antibodies block beta-amyloid fibril formation and prevent beta-amyloid-induced neurotoxicity. *Brain* 2003;126:1935–9.
 - [21] Dodel RC, Du Y, Depboylu C, Hampel H, Frolich L, Haag A, et al. Intravenous immunoglobulins containing antibodies against beta-amyloid for the treatment of Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2004;75:1472–4.
 - [22] Fang Q, Kannapell CC, Fu SM, Xu S, Gaskin F. VH and VL gene usage by anti-beta-amyloid autoantibodies in Alzheimer's disease: detection of highly mutated V regions in both heavy and light chains. *Clin Immunol Immunopathol* 1995;75:159–67.
 - [23] Hock C, Konietzko U, Papassotiropoulos A, Wollmer A, Streffer J, von Rotz RC, et al. Generation of antibodies specific for beta-amyloid by vaccination of patients with Alzheimer disease. *Nat Med* 2002;8:1270–5.
 - [24] Hock C, Konietzko U, Streffer JR, Tracy J, Signorell A, Muller-Tillmanns B, et al. Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. *Neuron* 2003;38:547–54.
 - [25] Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med* 2003;9:448–52.
 - [26] Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology* 2003;61:46–54.
 - [27] Ferrer J, Boada Rovira M, Sanchez Guerra ML, Rey MJ, Costa-Jussa F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. *Brain Pathol* 2004;14:11–20.
 - [28] Bayer AJ, Bullock R, Jones RW, Wilkinson D, Paterson KR, Jenkins L, et al. Evaluation of the safety and immunogenicity of synthetic Abeta42 (AN1792) in patients with AD. *Neurology* 2005;64:94–101.
 - [29] Bacskai BJ, Kajdasz ST, McLellan ME, Games D, Seubert P, Schenk D, et al. Non-Fc-mediated mechanisms are involved in clearance of amyloid-beta in vivo by immunotherapy. *J Neurosci* 2002;22:7873–8.
 - [30] DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM. Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science* 2002;295:2264–7.
 - [31] Solomon B, Koppel R, Frankel D, Hanan-Aharon E. Disaggregation of Alzheimer beta-amyloid by site-directed mAb. *Proc Natl Acad Sci U S A* 1997;94:4109–12.
 - [32] McLaurin J, Cecal R, Kierstead ME, Tian X, Phinney AL, Manea M, et al. Therapeutically effective antibodies against amyloid-beta peptide target amyloid-beta residues 4–10 and inhibit cytotoxicity and fibrillogenesis. *Nat Med* 2002;8:1263–9.
 - [33] Wilcock DM, DiCarlo G, Henderson D, Jackson J, Clarke K, Ugen KE, et al. Intracranially administered anti-A beta antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation. *J Neurosci* 2003;23:3745–51.
 - [34] Steinitz M, Klein G, Koskimies S, Makel O. EB virus-induced B lymphocyte cell lines producing specific antibody. *Nature* 1977;269:420–2.
 - [35] Steinitz M, Klein G. EBV-transformation of surface IgA-positive human lymphocytes. *J Immunol* 1980;125:194–6.
 - [36] Steinitz M, Tamir S, Ferne M, Goldfarb A. A protective human monoclonal IgA antibody produced in vitro: anti-pneumococcal antibody engendered by Epstein-Barr virus-immortalized cell line. *Eur J Immunol* 1986;16:187–93.
 - [37] Steinitz M. Human monoclonal antibodies produced by EBV immortalized cell lines: technical and theoretical principles. In: Pal RS, editor. *Immunoassay technology*. UK: Macmillan Press; 1988. p. 1–17.

- [38] Xu S, Gaskin F. Increased incidence of anti-beta-amyloid autoantibodies secreted by Epstein-Barr virus transformed B cell lines from patients with Alzheimer's disease. *Mech Ageing Dev* 1997;94:213–22.
- [39] Klein U, Rajewsky K, Kuppers R. Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. *J Exp Med* 1998;188:1679–89.
- [40] Traggiai E, Becker S, Subbarao K, Kolesnikova L, Uematsu Y, Gismondo MR, et al. An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. *Nat Med* 2004;10:871–5.

Prevalence of elevated anti-thyroid antibodies and antinuclear antibodies in children with immune thrombocytopenic purpura.

The prevalence and significance of autoantibodies found at the time of diagnosis of childhood ITP were studied to correlate their presence with risk for development of chronic ITP. Pratt EL, et al. (*Am J Hematol* 2005; 79: 175–9) tested children presenting with acute or chronic ITP at study entry and followed for the presence of anti-thyroid antibodies (ATA), antinuclear antibodies (ANA), Coombs' reactivity, and anti-human immunodeficiency virus (HIV) antibodies. Thirty-one patients were enrolled in the study with a median age of 8 years and male-to-female ratio of 1:1.8. Forty-two percent of these children had an acute course of ITP, and 58% of children had a chronic course of ITP. Of children with acute ITP, three (23%) had an acute nonplatelet autoantibody detected. Of the children with chronic ITP, six (33%) had at least one abnormal antibody value. Five children (16%) tested positive for ATA. Five children had positive ANA, and of these children, 4 (80%) had chronic ITP. No patients included in the study tested positive for HIV. These results suggest that patients with acute ITP who also have other autoantibodies may be more likely to develop chronic ITP than those lacking these autoantibodies.

Levels of complement in sera from inactive SLE patients, although decreased, do not influence in vitro uptake of apoptotic cells.

Complement factors facilitate the clearance of apoptotic cells and, when decreased, might result in an increased amount of apoptotic cells found in SLE patients. To determine the influence of complement profiles from inactive SLE patients on the in vitro phagocytosis of apoptotic cells, Grevink ME, et al. (*J Autoimmunity* 2005; 24: 329–36) studied 98 consecutive SLE patients with inactive disease (SLEDAI $\leq$ 4) and compared them with 20 healthy controls (HC). Levels of CH50, C3, C4, C1q, and C1r were measured. Human peripheral blood monocytes were isolated from healthy controls and cultured for 7 days to obtain monocyte-derived macrophages (MDM). Jurkat cells were irradiated with UVB to induce apoptosis. Phagocytosis was tested by incubation of MDM with apoptotic cells in the presence of serum and quantified as phagocytosis index. Serum from 20 patients with CH50 $<$ 65%, 20 patients with CH50 $\geq$ 65%, and 20 HC were used in this assay. All HC and 37% of patients had normal complement levels. CH50 level was decreased in 21% of patients, C3 in 52%, C4 in 29%, C1q in 2% and C1r in 44% of patients. Between patients and HC, differences in level of CH50, C3, and C4 were statistically significant. No correlation was found between the respective complement levels and phagocytosis index. In most SLE patients with inactive disease, levels of one or more complement components are decreased. However, decreased levels of complement do not result in a significantly reduced in vitro uptake of apoptotic cells by MDM.