# CONCLUSIONES

## OBJETIVO PRINCIPAL

-El timo humano expresa tránscritos de todas las cadenas que conforman el receptor de acetilcolina, de todos los autoantígenos involucrados en esclerosis múltiple excepto MOG, y de los antígenos prostáticos PSA y PSM

-Los autoantígenos del sistema nervioso central no guardan una expresión restringida como se pensaba, excepto para MOG, que es el único antígeno realmente secuestrado en el SNC

-Los autoantígenos presentan tres patrones diferentes de expresión intratímica, a saber:

*Variable*: con marcadas diferencias interindividuales en los niveles de expresión

*Constitutivo*: paralelo a la expresión de genes constitutivos, como GAPDH

*Dependiente del desarrollo*: expresión relacionada con la edad

## OBJETIVOS SECUNDARIOS

### OBJETIVO I

La expresión de los autoantígenos en el timo humano no guarda una relación directa con la edad, excepto para la cadena gamma del receptor fetal de acetilcolina.

### OBJETIVO II

Las formas de expresión de los autoantígenos en el timo humano son similares a las formas de expresión en el órgano diana, excepto PLP, que se expresa como una variante mas corta de *splicing* alternativo. Esta conclusión es válida para nuestro sistema de amplificaciones, pero no se descarta que en otras regiones de los genes de los autoantígenos existan formas de *splicing* no detectadas por nuestros cebadores.

### OBJETVO III

Los experimentos de detección de antígenos en las diferentes fracciones celulares y en las microdisecciones tímicas indican que las células responsables de la transcripción de los autoantígenos en el timo son las células epiteliales, predominantemente de localización medular.

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***Las células del Sistema Nervioso Central***

En el año 1846, Virchow describió que en el cerebro, además de las neuronas, existía otro tipo celular, y pensando de que se trataba del tejido conectivo, lo llamó *neuroglia*. En 1913 Ramon y Cajal y Rio Hortega, utilizando técnicas de impregnación con metales, caracterizaron dos tipos celulares que forman la neuroglia, los astrocitos y oligodendrocitos, y los llamaron *macroglia* (Ramón y Cajal, 1913.). Años mas tarde, Rio Hortega describió un tercer tipo celular y lo llamó *microglia,* para diferenciarlo de la *macroglia*, los astrocitos y los oligodendrocitos. Las células gliales constituyen el principal tipo celular del sistema nervioso central (el 90% en los seres humanos) y son necesarias para el desarrollo y la correcta función neuronal.

Los ***astrocitos***, transportan líquidos e iones hacia los capilares, y guían la migración celular durante la embriogénesis del sistema nervioso central. Desempeñan un papel estructural, además de formar la barrera hemato-encefálica rodeando a los endotelios capilares e impidiendo la circulación de las células sanguíneas en el sistema nervioso central (gráfico 13).

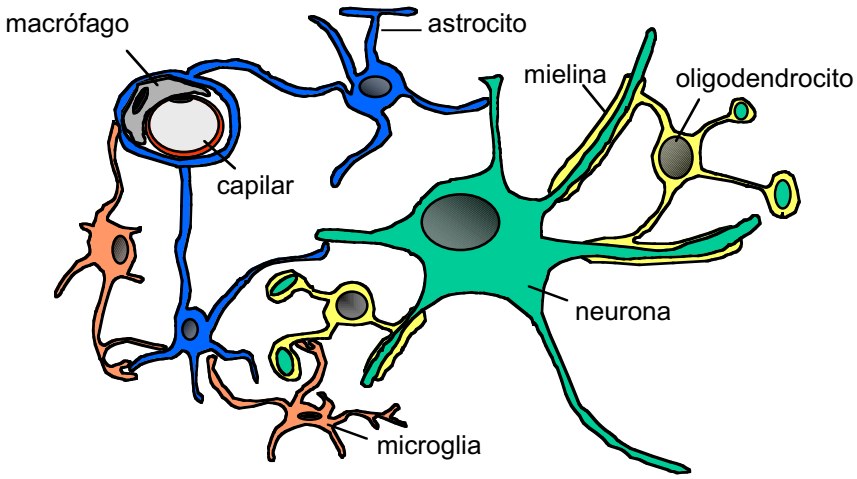


***Gráfico 13:*** *Representación de los tipos celulares en el sistema nervioso central.* Los astrocitos son células estrelladas, con numerosas prolongaciones que les permitan contactar con todos los demás tipos celulares, y forman la barrera hemato-encefálica, debido a que rodean a las células endoteliales de los capilares sanguíneos. La microglia son los macrófagos residentes del sistema nervioso central. Tienen capacidad de fagocitar restos celulares y después de un daño neural son capaces de activarse y de proliferar mitóticamente. Los oligodendrocitos son las células formadoras de la mielina.

La ***microglia***, son macrófagos especializados o células del sistema inmunológico en el sistema nervioso central (similares a las células dendríticas presentadoras de antígenos), debido a que tienen capacidad fagocítica y expresan moléculas de MHC de clase II.

Los ***oligodendrocitos***, son las células formadoras de la mielina. La vaina de mielina debe su nombre a Virchow (Virchow, 1854). Es una estructura espiral constituida por extensiones de la membrana plasmática de los oligodendrocitos en el sistema nervioso central, la cual se dispone de manera concéntrica alrededor de los axones, constituyendo una vaina multilaminar (gráfico 14).

Se compone de un 70% de lípidos, de un 30% de proteínas y de un bajo contenido en agua. Esta proporción es inversa a la composición normal de la membrana plasmática, pero es acorde con la función de conducción del impulso nervioso, a alta velocidad y a largas distancias.

Todos los constituyentes de la mielina son sintetizados por el oligodendrocito. Los lípidos que constituyen la mielina son colesterol, fosfolípidos y glicolípidos en una proporción aproximada de 4:3:2. Los componentes proteicos mas importantes de la mielina son la proteína básica de mielina (MBP) y la proteína proteolipídica (PLP), que son proteínas de bajo peso molecular y representan el 80% de las proteínas mielínicas (Campagnoni, 1988). La mielina también contiene otros componentes proteicos minoritarios, como la glicoproteína asociada a la mielina (MAG), y la glicoproteína mielínica de los oligodendrocitos (MOG).

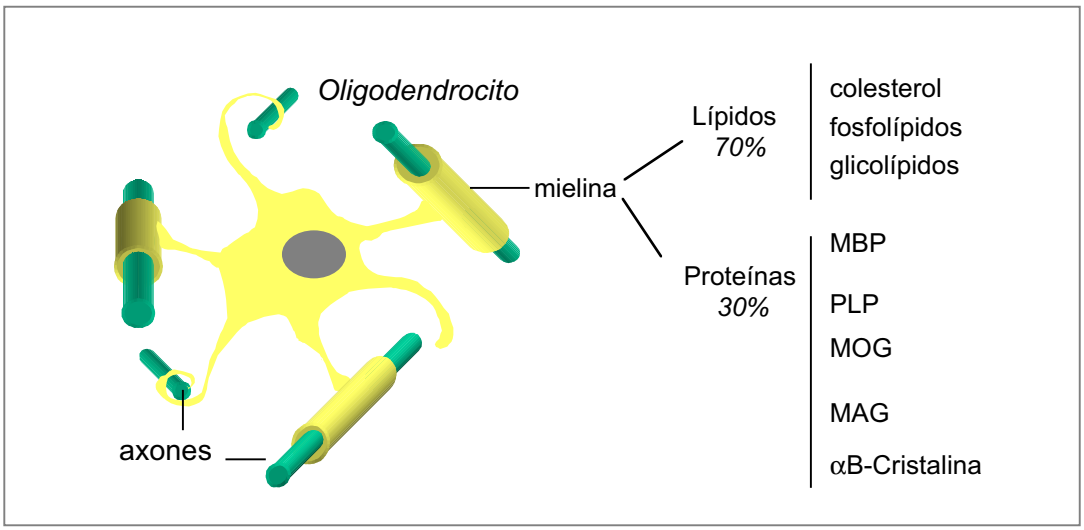


***Gráfico 14:*** *Componentes de la vaina de mielina del sistema nervioso central*

***Antígenos mielínicos***

***Proteína Proteolipídica (PLP)***

La PLP es uno de los principales componentes proteicos de la mielina, ya que representa casi el 50% de las mismas. Recibe el nombre de proteína proteolipídica debido a que en el año 1951, Folch extrajo gran cantidad de proteínas del cerebro empleando mezclas de solventes orgánicos y pensando que se trataba de complejos proteolipídicos, las denominó "*proteolípidos"* para diferenciarlas de otras lipoproteínas solubles en agua (Folch and Lees, 1951).

La PLP del sistema nervioso central existe en dos isoformas: la isoforma predominante llamada PLP *full-length* (de 25-kD) y la isoforma corta, llamada DM20 (de 20-kD). Ambas isoformas son codificadas por el mismo gen de 15 Kb (Nave et al., 1986) localizado en el cromosoma X en la posición Xq22 en humanos, y en el área H2C en ratones (Willard and Riodan, 1985). El gen comprende 7 exones, que codifican el mRNA de la forma full-length de PLP, mientras que la isoforma DM-20, es el resultado de *splicing* alternativo del mRNA, que pierde parte del exón 3 (exón 3B) y resulta en la deleción de 35 aminoácidos de la secuencia proteica.

Existe un 100% de homología en la secuencia peptídica de PLP de ratón y la humana (Diehl et al., 1986). La estructura secundaria de la proteína comprende dos dominios extracitoplasmáticos, cuatro dominios de transmembrana, y tres dominios intracitoplasmáticos que incluyen los extremos N y C-terminal (gráfico 15).

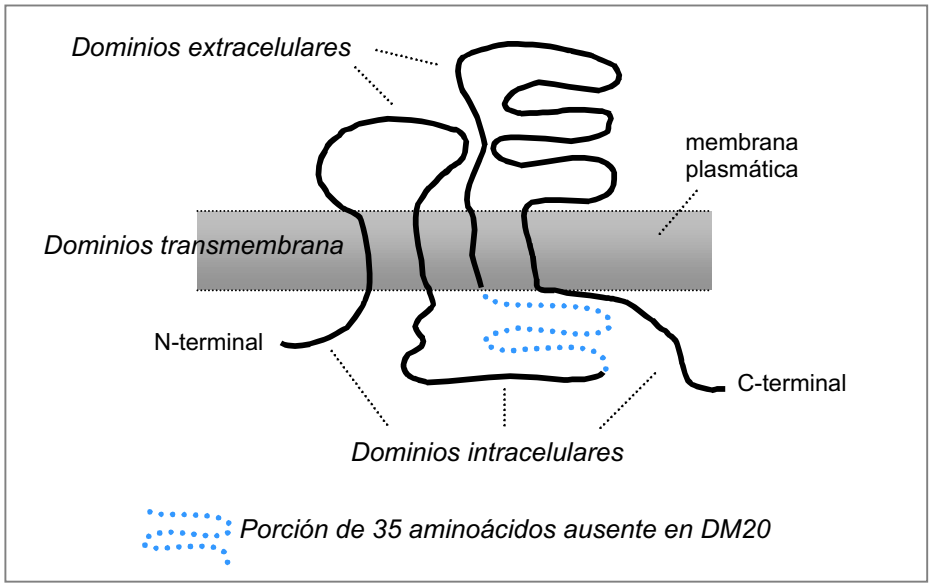


***Gráfico 15:*** *Representación de la estructura secundaria de PLP*

La función de PLP en la vaina de mielina es la de estabilizar las uniones de la membrana (Boison et al., 1995; Klugmann et al., 1997). En los ratones knock-out de PLP, que no expresan PLP ni DM20, se observa que si bien los oligodendrocitos son capaces de mielinizar a los axones, la vaina de mielina carece de estabilidad (Boison and Stoffel, 1994).

***Glicoproteína mielínica de los oligodendrocitos (MOG)***

MOG, es una proteína específica de los oligodendrocitos localizada en la superficie exterior de la vaina de mielina (Brunner et al., 1989). Es un componente minoritario de las proteínas mielínicas, ya que representa solo el 0,01-0,05 % en peso de las proteínas de membrana (Linington et al., 1984).

MOG es una proteína de 218 aminoácidos que pertenece a la superfamilia de las inmunoglobulinas (Gardinier et al., 1992) y está codificada dentro de la región distal del MHC (Pham-Dinh et al., 1993). El gen MOG humano tiene un tamaño de 11.1 Kb y se organiza en 10 exones. Los tránscritos dominantes de MOG, sin embargo, están codificados por 8 exones homólogos (Hilton et al., 1995). La proteína resultante es una glicoproteína de membrana de tipo I (ver gráfico 16), de 26-28 kD, que presenta un dominio Ig en el extremo N-terminal, un dominio transmembrana y un tercer dominio integrado con la cara interna de la membrana plasmática (della Gaspera et al., 1998; Kroepfl et al., 1996).

La función de MOG no se conoce, pero dada la localización extracelular de su dominio N-terminal en la vaina de mielina (Brunner et al., 1989), algunos autores sostienen que podría actuar como receptor, mientras que otros piensan que desempeña un papel estructural en el mantenimiento de la mielina, a través de interacciones con las proteínas de la matrix extracelular. El extremo C-terminal también parece ser funcionalmente importante en interacciones intracelulares, ya que es un dominio altamente conservado en diferentes especies (Gardinier et al., 1992; Pham-Dinh et al., 1993).

Desde el punto de vista inmunológico, MOG es un importante autoantígeno en las enfermedades desmielinizantes del sistema nervioso central. En el modelo animal de esclerosis múltiple, EAE, la inoculación de péptidos de MOG provoca enfermedad desmielinizante, como consecuencia de la respuesta inmune humoral (Bernard et al., 1997; Ichikawa et al., 1999). Además, en ausencia de anticuerpos específicos contra MOG, la patología en los modelos animales es solamente inflamatoria, mientras que, por el contrario, la inyección de un anticuerpo monoclonal específico contra MOG al inicio de la enfermedad produce una extensiva desmielinización, aumenta la respuesta inflamatoria y la severidad de la enfermedad (Iglesias et al., 2001).

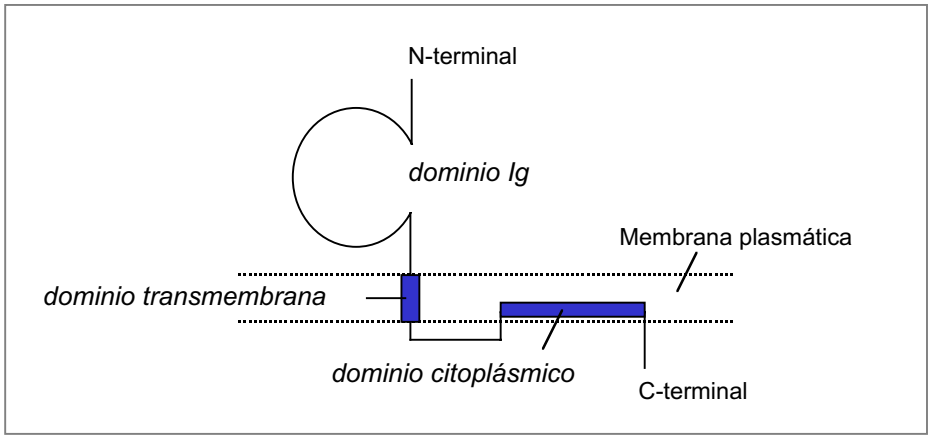


***Gráfico 16:*** *Representación de la estructura secundaria de MOG*

### α ***B Cristalina***

α-Cristalina es un miembro de la familia de proteínas pequeñas del shock térmico (*sHSP*) (Klemenz et al., 1991), y constituye un agregado de dos polipéptidos, αA- y αB-Cristalina, de 20-kDa cada uno, que comparten un 55% de homología y que forman agregados heterogéneos solubles, en proporción 3:1. α-Cristalina es la principal proteína soluble del cristalino del ojo, y tiene una importante función en el establecimiento del índice de refracción del citoplasma. Sin embargo, sus funciones celulares no se restringen al cristalino debido a su expresión ubicua, la actividad de autokinasa, la asociación con enfermedades neurodegenerativas y la capacidad de proteger contra el golpe térmico impidiendo la agregación proteica. De las dos subunidades que la constituyen, αA-Cristalina se encuentra algo mas restringida al cristalino, en tanto que αB-Cristalina es más ubicua y su expresión se puede inducir por stress.

αB-Cristalina se ha encontrado expresada en altas concentraciones en el citoplasma de los oligodendrocitos y de los astrocitos en las lesiones desmielinizantes en la esclerosis múltiple, y se la considera por ello un antígeno mielínico. Además, en las lesiones activas de la esclerosis múltiple, caracterizadas por desmielinización y presencia de infiltrado inflamatorio, se puede detectar αB-Cristalina entre las proteínas fagocitadas por los macrófagos, lo cual sugiere que la αB-Cristalina es probablemente procesada y presentada como antígeno al sistema inmune celular (Bajramovic et al., 2000).

***Antígenos no mielínicos***

***S100 β***

S100β es una proteína que une el calcio y que se encuentra abundantemente expresada en los astrocitos (Baimbridge et al., 1992). Forma parte de una familia de proteínas ligadoras de calcio constituida aproximadamente por 16 miembros, cada uno de los cuales presenta un patrón de expresión específico de tejido. Aunque la distribución de esta familia de proteínas no se encuentra restringida al SNC algunos miembros como S100α y S100β actúan en el SNC como proteínas moduladoras del calcio intracelular y uniendo estímulos extracelulares con respuestas intracelulares a través de la interacción con otras proteínas citoplasmáticas (Zimmer et al., 1995), como por ejemplo neurocalcin delta (Okazaki et al., 1995).

S100β está codificada en el cromosoma 21. Presenta 3 exones y dos intrones (Allore et al., 1990) y los exones 2 y 3 codifican dos dominios independientes de unión al calcio. Aunque el significado funcional de esta proteína no está claro, se ha comprobado que contribuye al funcionamiento normal del hipocampo. Es una proteína pequeña y soluble, de 91 aminoácidos (Jensen et al., 1985), que representa un 0,4% del total de las proteínas solubles del SNC (Moore, 1965). Su expresión en el SNC está restringida a los astrocitos, quienes además secretan una forma biológicamente activa de S100β que puede ser detectada en el líquido cefaloraquídeo. En el sistema nervioso periférico se encuentra expresada en las células de Schwann, y en las células de Müller en la retina.

## *Antígenos prostáticos*

### *Antígeno Prostático específico (PSA)*

El PSA es una glicoproteína de 240 aminoácidos, con un peso molecular de 28-30 KDa. Se sintetiza en el epitelio prostático y se secreta como un constituyente normal del fluido seminal. Pequeñas cantidades son secretadas a la sangre, y por ello es posible detectarlo en suero.

Su función biológica es enzimática, es una serinoproteasa y la mayor proporción de PSA circulante en el suero se encuentra unida a inhibidores de proteasas endógenos, como la $\alpha$-1 antiquimotripsina y la $\alpha$-2 macroglobulina. El PSA se expresa en altos niveles en el tejido prostático pero en bajos niveles en otros tejidos.

El gen que codifica para PSA tiene 5 exones. Su expresión está regulada por andrógenos: la transcripción del PSA comienza cuando el receptor de andrógenos, activado por la unión de su ligando, se une a la región promotora del gen PSA (Cleutjens et al., 1997)

Líneas celulares de origen no prostático, células sanguíneas y células de la médula ósea pueden expresar bajas cantidades de RNA de PSA (Smith et al., 1995).

### *Antígeno Prostático Específico de Membrana (PSM)*

El PSM es una glicoproteína de transmembrana de tipo II, de 84,3 KDa constituida por 750 aminoácidos que presenta tres dominios estructurales: un dominio C-terminal extracelular (de 707 aminoácidos), un dominio transmembrana (de 24 aminoácidos) y un dominio N-terminal intracelular (de 19 aminoácidos) (Gregorakis et al., 1998). La secuencia presenta un 54% de homología con el mRNA del receptor de transferrina humano (Israeli et al., 1993).

El gen que la codifica se localiza en el cromosoma 11, el DNA genómico tiene un tamaño de 93525 pb y se organiza en 19 exones y 18 intrones. Su expresión está aumentada por bFGF, TGF-$\alpha$ y EGF (Israeli et al., 1994a) y disminuida por andrógenos como la 5$\alpha$-DHT (Israeli et al., 1994b).

Su función biológica es enzimática, es una $\alpha$-dipeptidasa expresada en la superficie de las células epiteliales prostáticas (Gregorakis et al., 1998). Se utiliza como marcador específico del epitelio prostático ya que las células basales son negativas para PSM.

También se ha detectado expresión de PSM fuera de la próstata, en la mucosa duodenal, los túbulos renales y en linfocitos de sangre periférica (Lintula and Stenman, 1997).

En tejido prostático normal existe además una variante mas corta resultante de splicing alternativo, llamada PSM`. PSM´ ha perdido 266 nucleótidos del extremo 5`del cDNA que codifican para el codón de iniciación de la traducción y codones que codifican para 57

aminoácidos que forman parte del dominio de transmembrana de PSM. La proteína resultante es una proteína de 693 aminoácidos, con un peso molecular de 78 KDa, de localización citosólica (Su et al., 1995).

Aun no está clara la consecuencia biológica de estas variantes de *splicing* en la próstata, pero se ha demostrado que en los tumores primarios de próstata, el PSM es la forma predominante, mientras que en la próstata normal, la forma corta PSM´ es la predominante (Su et al., 1995).

## PUBLICACIONES

Los resultados descritos en esta memoria están siendo preparados para su publicación:

Bruno R, Tolosa E, Sospedra M, Fancesc-Ferrer X, Melms A, Foz-Sala M, Coll-Cantí J and Pujol-Borrell R.

*Transcription of Nicotinic Acetylcholine Receptor subunits in human thymus: three patterns of expression emerge.*

Bruno R, Sospedra M, Sabater L, Francesc-Ferrer X, Escudero D, Martínez-Cáceres E and Pujol-Borrell R.

*Multiple Sclerosis candidate autoantigens except MOG are transcribed in human thymus.*

Otras publicaciones relacionadas con esta tesis:

Sospedra M, Ferrer-Francesch X, Dominguez O, Juan M, Foz-Sala M, Pujol-Borrell R. Transcription of a broad range of self-antigens in human thymus suggests a role for central mechanisms in tolerance toward peripheral antigens. J Immunol 1998; 161: 5918-29.