UNIVERSIDADE BEIRA INTERIOR

ON THE ROLE OF MHC-I MOLECULES ON COGNITIVE IMPAIRMENT AND DEMENTIA: A NON-IMMUNE APPROACH

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On the role of MHC-I molecules on cognitive impairment and dementia:
A non-immune approach

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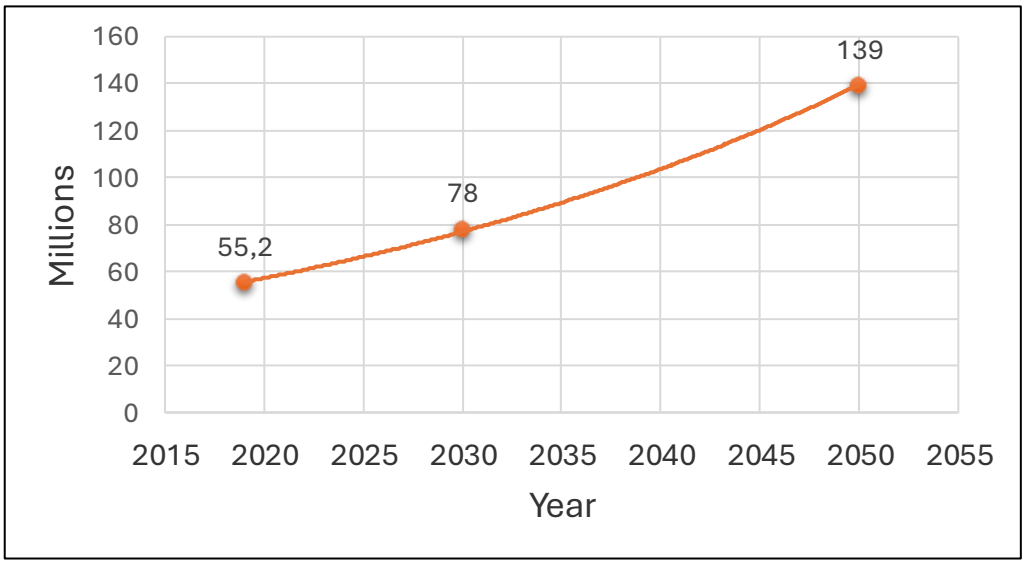
Preface

As mankind breaks the boundaries of potential years to live, the process of aging imposes various cellular challenges. Cells become less capable at repairing themselves and, as a result, damage accumulates. Furthermore, there is impaired protein formation. It causes a constant state of chronic low-level inflammation on tissues such as the brain, resulting in neuroinflammation. Persistent chronic neuroinflammation can harm neurons, contributing to the development of neurodegeneration, a pathological process that affects cognitive function, causes its impairment, and is often reflected by dementia. Because there is no treatment, it results in a decline in quality of life and an increase in medical costs that involves the continuous management of people suffering from these types of disorders. Following the World Health Organization report on the public health response to dementia (1), it was estimated that in 2019 nearly 55 million people worldwide were living with dementia and by 2050 this number is projected to skyrocket to a staggering 139 million.

Figure 1. Projection for Number of People with Dementia until 2050*



*Adapted from Global status report on the public health response to dementia. https://www.who.int/publications/i/item/9789240033245 (accessed, Jun 3, 2023)

This projection underscores the urgent need to address the impact of neurodegeneration and reduce its burden. One approach to prevent these diseases is to try to understand their onset and do an early diagnosis. It is

critical to concentrate on preventative interventions that might possibly postpone or even prevent the beginning of neurodegenerative illnesses. Early detection is essential in this context since it allows for prompt intervention and the adoption of relevant medications or lifestyle changes.

This thesis work is based on the premise that the MHC class I molecules (MHC-I) present in the plasma membrane of all nucleated cells, through their capacity to exist in a physiological equilibrium between forms consisting of a heavy chain (αHC), a light chain (β2micoglobulin, β2m) and an 8-12 aminoacid peptide (called closed conformers) and forms consisting only of the αHC, after the loss of the β2m and the peptide (called open conformers), are gate-keepers of regulating the intracellular signals transmitted by the binding of nutrients and hormones to their receptors.

Understanding the influence of these polymorphic molecules on aging and neurodegeneration can open new avenues of research aiming at preserving cognitive function and ultimately to reduce the prevalence and the detrimental effect of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Thus, MHC-I molecules, their molecular partners and the signaling pathways they modulate may become biomarkers of cognitive decline and, at the same time, putative targets for the development of innovative immunotherapies.

To elaborate this thesis work, a scientific search for relevant articles was carried out using the *PubMed* database and using combinations of the following keywords*:* aging, Alzheimer's disease (AD), β2-microglobulin (β2m), brain, central nervous system (CNS), cognitive impairment, dementia, major histocompatibility complex class I (MHC-I), misfolded, free heavy chains, open conformers, peptide-empty, empty conformers, neurogenesis, neurodegeneration, neuro plasticity, neuro regeneration, human leukocyte antigens class I (HLA-I) and Parkinson's disease (PD). Initially, 205 articles were selected.

After establishing inclusive and exclusive criteria (see Table 1), forty articles were removed. Even though, initially, it was our purpose to exclude Review

articles, this criterion was reviewed considering that the area covered in this thesis work has been overlooked or even neglected for many years. Therefore, and due to the seminal character of some of these reviews in describing basics aspects of the biology and physiology of MHC-I molecules unrelated with a classical immunological response, and because they recapitulate the scattered reports published over the past 35 years on the significance of MHC-I molecules in the regulation of non-immunological processes, a number of original and review articles were included for analysis and discussion. In summary, a total of 177 articles were used for the preparation of this thesis.

Table 1. Exclusive and Inclusive Criteria

Exclusion Criteria (NOT)	**Inclusion Criteria (AND)**
Review	Journal
Clinical Trials	Original Articles
Multiple Sclerosis	
Human Immunodeficiency Virus	
Ankylosing Spondylitis	

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Resumo

Este trabalho tenta destacar a influência das moléculas do Complexo Principal de Histocompatibilidade Classe I (MHC-I) na homeostase do sistema nervoso central (SNC) e como anomalias na sua expressão podem levar ao deterioramento cognitivo. Investigações iniciais relacionadas com a função cerebral revelaram que este órgão não expressava moléculas MHC-I, não era reconhecido por linfócitos T CD8+ e, por isso, era imunopriveligiado. Contudo, estudos recentes não só demonstraram que neurónios e outras células do sistema nervoso central (CNS) expressavam moléculas MHC-I, como também estas moléculas desempenham um papel crucial na formação, função e modelação das sinapses do CNS no período embrionário, no nascimento e na idade adulta, nomeadamente durante condições inflamatórias. A quantidade de investigação disponível nesta área sugere que as moléculas MHC-I e as vias de sinalização que por elas são reguladas podem esclarecer sobre os mecanismos moleculares e celulares responsáveis por regular a homeostase cerebral em condições fisiológicas e patológicas. Desta forma, estas moléculas podem tornar-se potenciais biomarcadores da deterioração cognitiva e alvos para imunoterapias inovadoras.

Palavras-chave

Antigénios Leucocitários Humanos;β2-microglobulina;Cérebro;Complexo Principal de Histocompatibilidade Classe I;Estructuras de MHC-I abertas; Demência;Deterioração Cognitiva;Doença de Alzheimer;Doença de Parkinson; Envelhecimento;Sistema Nervoso Central

Abstract

This work tries to recapitulate the influence that Major Histocompatibility Class I (MHC-I) Molecules have on the homeostasis of the central nervous system (CNS) and how abnormalities in its expression can lead to cognitive deterioration. Initial research into brain function revealed that this organ did not express MHC-I molecules, could not be recognized by cytotoxic CD8+ T cells and, hence, was immunoprivileged. However, studies carried out during recent years not only demonstrated that neurons and other central nervous system (CNS) cells express MHC-I molecules, but that these molecules play essential roles in the establishment, function, and modeling of synapses in the CNS during the embryonic period, at birth and during adulthood, namely during inflammatory conditions. The accumulated body of evidence suggests that MHC-I molecules and the signaling pathways they regulate could provide clues on the molecular and cellular mechanisms regulating brain homeostasis in health and disease, thus becoming potential biomarkers of cognitive decline and targets for innovative immunotherapies.

Keywords

Aging;Alzheimer's Disease;β2-microglobulin;Brain;Central Nervous System;
Cognitive Impairment;Dementia;Major Histocompatibility Complex Class I;
Open MHC-I Conformers;Human Leukocyte Antigens Class I;Parkinson's
Disease

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List of Abbreviations

Aβ	Amyloid Beta
AD	Alzheimer's Disease
APC	Antigen Processing Cell
ApoE	Apolipoprotein E
Arf	ADP-Ribosylation Factor
BBB	Blood Brain Barrier
cAMP	Cyclic Adenosine Monophosphate
CD3ζ	CD3 Zeta
CNS	Central Nervous System
CNX	Calnexin
CRT	Calreticulin
CSF	Cerebrospinal Fluid
CTL	Cytotoxic T Lymphocyte
DD	Disordered Domain
EBI	Elderly from Beira Interior
EEA-1	Early Endosome Antigen 1
EHD-1	EH Domain Containing 1
ER	Endoplasmic Reticulum
ERAD	Endoplasmic Reticulum-Associated Degradation
ERAP1	Endoplasmic Reticulum Amino Peptidase 1
ERC	Endocytic Recycling Compartment
GTP	Guanosine-5'-triphosphate
GTPase	Guanosine-5'-triphosphase
HFE	High Fe^{2+}
HLA-I	Human Leukocyte Antigen class I
IFN	Interferon
IL	Interleukin
KIR	Killer Cell Ig-like Receptor
Lamp-1	Lysosomal Associated Membrane Protein 1
LAP	Leucine Amino Peptidase
LILR	Leukocyte Ig-like Receptor
MHC	Major Histocompatibility Complex
MHC-I	Major Histocompatibility Complex class I
MHC-II	Major Histocompatibility Complex class II
MICAL-L1	Molecule Interacting with CasL-Like 1
mRNA	Messenger Ribonucleic Acid
NK	Natural Killer
PD	Parkinson's Disease
PirB	Paired Immunoglobulin-Like Receptor B
PLC	Peptide Loading Complex
pS335	Phosphorylated Serine Residue 335
pSNAP-23	Phosphorylated Synaptosomal-Associated Protein 23
pY320	Phosphorylated Tyrosine Residue 320
Rab	Ras-Associated Binding

RNA	Ribonucleic Acid
S335	Serine Residue 335
sMHC-I	Soluble Major Histocompatibility Complex class I
TAP	Transporter Associated with Antigen Processing
TCR	T Cell Receptor
TLR	Toll-Like Receptors
TNF	Tumor necrosis factor
TRE	Tubular Recycling Endosomes
VAMP-8	Vesicle-Associated Membrane Protein 8
WT	Wild-Type
Y320	Tyrosine Residue 320
αHC	Alpha Heavy Chain
β2m	Beta2-Microglobulin

Chapter 1 – HISTORICAL PERSPECTIVE OF MHC CLASS I MOLECULES

The Major Histocompatibility Complex (MHC; HLA in humans; H-2 in mice) was initially identified as a chromosomal region located in the short arm of the chromosome 6 (6p21.3), responsible for accepting or rejecting transplanted tissues in mice. Molecules of the Major Histocompatibility Complex were named after this genetic system (2). MHC is the most polymorphic region in the human genome at the time of writing. Within this same region, two main classes of MHC molecules are encoded: MHC class I (thereafter MHC-I) and MHC class II (thereafter MHC-II). Both classes can be divided into classical and non-classical. In humans, classical HLA class I (thereafter HLA-I) molecules are divided into three main types: HLA-A, HLA-B, and HLA-C, are highly polymorphic and with a wide tissue distribution. In contrast, non-classical HLA-I molecules (HLA-E, HLA-F and HLA-G) are less polymorphic and with a restricted tissue distribution (3). This thesis work will concentrate on the classical MHC-I/HLA-I/H-2 molecules.

MHC-I molecules are defined as a trimeric structure made up of a heavy chain (thereafter, αHC), a light chain (beta2-microglobulin; abbreviately β2m) and by an 8-12 aminoacid peptide. The extracellular part is divided into three domains: α1, α2 and α3. The domains α1 and α2 are responsible for the high polymorphism characteristic of this molecule, and they both create the groove where the peptide attaches before the MHC-I molecule is transported to the plasma membrane, where eventually will be recognized by CD8+ T cells and initiate an immune response against intracellular pathogens and tumoral cells (4,5).

Although the most studied role of MHC-I molecules was in the immune response, MHC-I molecules have intrigued many researchers since their discovery because, in addition to their classic and main role as a peptide presenting structure to CD8+ T cells, they have other overlooked functions. Indeed, initial studies carried out during the 1970s mostly in mice by several investigators revealed a number of major non-immunological traits that were affected by the MHC-I genotype. These traits were compiled by Meruelo & Edidin in a review published in 1980 (6), and included:

1. Susceptibility or resistance to diseases
2. Levels of plasma testosterone
3. Weights of steroid-sensitive organs
4. Mating behavior
5. Cell adhesion
6. Liver cAMP levels
7. Binding of hormones to cell membranes

Unfortunately, these seminal observations had very little impact on the ensuing research in the area of immunology, which was dominated by studies of the immune response against microbes. However, the investigators that unveiled these "liaisons" left the future scientists with daring proposals on the non-immunological roles of MHC-I molecules. Thus, the MHC associations with susceptibility or resistance to disease, which resulted from early studies showing a clinical correlation between the presence of the HLA-I molecule HLA-B27 (formerly designated HLA-W27 and HLA-A27) and the onset of ankylosing spondylitis (7), led, in 1976, to the hypothesis by the Danish researchers Svejgaard and Ryder that MHC-I molecules on the cell surface could interfere with ligand-receptors interactions that were not directly related to immune responses (8). They postulated that, under specific conditions, lateral interactions between HLA-I molecules and the receptors could cause disease and provide an explanation for some of the connections between certain HLA-I alleles and non-immunological diseases in humans at that time, such as hereditary hemochromatosis and manic-depressive disease. This postulate was proven correct for the inherited iron overload disease hemochromatosis 20 years later when the gene for hemochromatosis, *hfe*, was found to encode for a non-classical MHC-I molecule called HFE (for High Iron) that lacks peptide but binds β2m (9). It is now known that HFE directly influences iron absorption by physically *cis*-associating with the transferrin receptor lowering its affinity for transferrin (10,11). Nowadays, it is also known that HFE influences iron absorption indirectly by up-regulating hepcidin expression, the main regulator of iron metabolism (12).

Regarding the genetic control of the binding of hormones to their receptors and the subsequent generation of intracellular levels of cAMP, it is worth noting pioneering studies revealing that the biding of glucagon and insulin to their receptors present in liver membranes was inhibited by anti-HLA antibodies (13,14). These results, to

which we will return later, suggested that MHC-I molecules were possibly involved in the regulation of hormone function by molecular *cis*-association with the receptors, something demonstrated later for the insulin receptor (15).

Last, but not least, and based on the above referenced traits that were affected by the MHC-I genotype, it is important for this thesis to mention a postulate put forward by Susumu Ohno in 1977. According to his theory, the initial function of surface MHC-I molecules was to act as anchors for regulating plasma membrane proteins that participated in cell differentiation and organogenesis (16). His essay hypothesized that, in the normal course of development, the interactions between MHC-I molecules and their corresponding receptors on the cell plasma membrane likely functioned as the internal signal necessary for triggering differentiation through cell-cell association. Preliminary experimental evidence suggesting the potential involvement of MHC-I molecules in the control of cell growth and organ development emerged a decade later. These findings were based on studies revealing that introducing the murine H-2L^d gene into a human colon carcinoma cell line resulted in the inhibition of anchorage-independent growth and tumor formation in immunodeficient mice (17). Conversely, transfection of the same H-2L^d gene into a human neuroendocrine carcinoma cell line had the opposite effect, that is, it increased the formation of metastatic lung colonies in immunodeficient mice (18). In this regard, it is important to note that the murine H-2L^d molecules are more prone than other H-2 molecules to lose the β2m and the peptide and become open conformers, an aspect of the biochemistry of MHC class I molecules that will be covered in more depth later (19). These features, when combined, could explain the differences observed in the two cell lines and illustrate the complexity of the signaling pathways that control cell growth and differentiation.

The purpose of this historical perspective was to introduce MHC-I molecules not only as structures involved in the activation of CD8+ T cells but also as key players in the regulation of basic physiological functions of the cells. Among these, one is worth mentioning: the regulation of neuronal development and function. Although the molecular mechanisms used are poorly characterized and misunderstood, the past and present body of knowledge suggest that these mechanisms are likely related to the capability of MHC-I molecules to interact in *cis* and *trans* with signaling receptors important for normal neuronal function. Of note, recent studies in the area

of neurobiology have conclusively proven Ohno's hypothesis by showing, in mice models, that MHC-I molecules are involved in the regulation of neurogenesis and neuronal plasticity (20–22). Therefore, this thesis will focus on the influence that MHC-I molecules have on CNS homeostasis and how anomalies in the expression of these molecules may be associated with neurodegeneration. This will bring to a medical audience, normally oblivious to this knowledge, the importance of the influence that MHC-I molecules have on cognitive disfunction, which may lead to neurodegeneration and dementia.

Chapter 2 – BIOLOGY OF MHC-I MOLECULES

2.1. Structure and cell surface expression

The genes responsible for encoding the αHC are located on the chromosome 6 and they consist of eight different exons. Exon 1 encodes the initial sequence, or leader peptide, responsible for moving the initial synthesis of the MHC-I molecule to the endoplasmic reticulum (ER). Exons 2, 3 and 4 encode the extracellular part, consisting of the three domains: α1, α2 and α3. Exon 5 encodes for the transmembrane region. Finally, exons 6 and 7 encode for the cytoplasmatic tail. The gene that encodes for the β2m light chain is located outside the MHC region, on chromosome 15 (4).

The synthesis of the αHCs occurs in the cytoplasm and continues on the ER (23) (see Figure 2). The passage to this cellular compartment is mediated by the transporter Sec61. Once in the ER, the αHC associates with a chaperon named Calnexin, whose main function is stabilizing their formation. In the absence of Calnexin, an alternative chaperone, the Immunoglobulin Binding Protein, takes its place. After performing its role, Calnexin is replaced by Calreticulin and Endoplasmic Reticulum-resident protein 57. These chaperones allow the formation of disulfide bons within the α2 and α3 heavy chains and allow interaction with the β2m light chain. After this process, the heterodimer αHC-β2m associates with the chaperone Tapasin and with the Transporter Associated with Antigen Processing (TAP), leading to the formation of the Peptide Loading Complex (PLC) (23). The main function of PLC is to hold the αHC-β2m complex on the ER until a peptide, that confers stability, is attached to it.

The peptides are generated by the proteosome, a multienzymatic complex present in the cytosol of the cell that breaks-down proteins signalized by ubiquitin, a regulatory protein, into 5 to 12 aminoacid peptides that are then carried into the ER lumen through TAP. These aminoacids formed by the proteosome can have their binding affinity to the MHC-I molecule enhanced by the presence of IFN-γ. Furthermore, the proteosome can originate some peptides that may need to undergo through additional proteolysis, which can be performed by the Leucine Amino Peptidase (LAP) present in the cytosol of the cell, or by the ER Amino Peptidase 1 (ERAP1)

present in ER lumen. The activity of ERAP1 can be increased by IFN-γ (5). Besides the aminoacids formed by the proteosome, recently synthesized proteins, such as Defective Ribosomal Products or peptides derived from viral proteins, can also be transported by TAP. If the MHC-I molecule does not have a peptide attached or if it does not acquire the correct conformation, it is sent by Sec61 from the ER back to the cytosol to be recycled. This process, named ER-Associated Degradation (ERAD), controls de quality of the production of MHC-I molecules (5,23).

When the MHC-I molecule is finally fully assembled (αHC+β2m+peptide), it acquires its classical *folded* conformation, detaches from the PLC, and it is transported to the Golgi cisterns. The chaperones Tapasin, Calreticulin and Endoplasmic Reticulum-resident protein 57 remain in the ER, for further formation of other MHC-I molecules (23). In the Golgi, the α1 domain of the heavy chain of the MHC-I molecule is glycosylated to prevent its destruction by H endoglycosidase, an enzyme that verifies if a molecule went through a correct mature process in the ER (23). Finally, the MHC-I molecule is transported to the cell membrane where, during its lifespan of 6-7 hours, will be eventually recognized by the T cell receptor (TCR) of naïve CD8+ T cells or by killer cell Ig-like receptors (KIR) and leukocyte Ig-like receptors (LILR) expressed by NK cells and effector-memory CD8+ T cells (thereafter CD8+ TEM) (24).

Figure 3. Schematic representation of the formation of MHC-I molecules (Adapted from (25,26)).

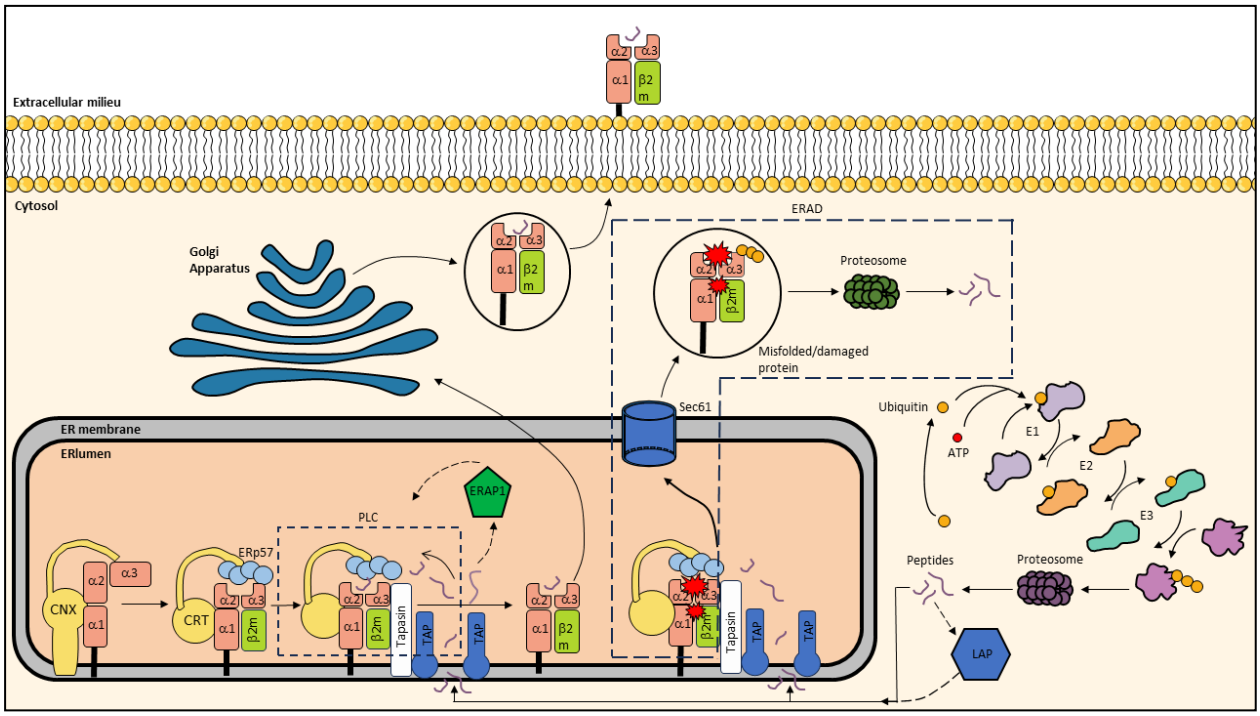


Figure 2 Formation of MHC-I molecules. Once the α heavy chains 1,2,3 enter the Endoplasmic Reticulum (ER) by Sec61 (not shown) they associate with Calnexin (CNX). Then, CNX is replaced by Calreticulin (CRT) and ERp77, allowing the α heavy chains to engage with β2m. From here, the α heavy chains-β2m associate with Tapasin and the Transporter Associated with Antigen Processing (TAP), forming the Peptide Loading Complex (PLC). Meanwhile, a proteosome located in the cytosol breaks down ubiquitin-signaled peptides. These peptides may require further degradation by the Leucine Amino Peptidase (LAP). TAP transports the allocated proteins into the ER lumen. In the ER, peptides may need to be further broken down by the Endoplasmic Reticulum Amino Peptidase 1 (ERAP1). These peptides then associate with PLC. From here, the fully assembled MHC-I molecule is formed (αHC+β2m+peptide), it detaches from the various chaperones and is transported from the ER to the Golgi Apparatus, and eventually to the cell membrane. During this step, the MHC-I molecule is examined to see if it was formed correctly. If the MHC-I molecule does not acquire its folded state (e.g., becomes misfolded) or does not have a peptide linked with it, it is sent from the ER to the cell cytosol by Sec61 and signaled by ubiquitin to be degraded by other proteosomes in a process known as Endoplasmic Reticulum-Associated Degradation (ERAD)

Expression of MHC-I molecules is regulated through the activation of the enhanceosome, a group of DNA regulatory sequences that includes *enhancer A*, *IFN-Stimulated Response Element*, the *SXY module* and, more recently, the *Class I Trans-Activator protein,* NOD-Like Receptor Family CARD domain containing 5 (27)**.** This group of sequences are activated by different extracellular signals, namely cytokines. The most important cytokines for this process are IFN-γ and TNF-α, however other cytokines such as IFN-α, IFN-β and IL-4 also have some degree of influence (28). Based on an extensive review conducted by Zhou et al. (29), a summary of the effect of IFN-γ on MHC-I expression on different cell types is listed in Table 2.

Table 2. Effects of IFN-γ on MHC-I expression in different types of cells

Cells and/or Organ	Effect of IFN-γ
CD4+ T cells	• Increased MHC-I surface expression
Monocytes	• Upregulation of TAP2 and TAP1
Endothelial cells	• Increased TAP expression and peptide transport • Increased MHC-I heavy chain expression
β-pancreatic cells	• Activation MHC-I antigen processing and presentation • Increased MHC-I expression on cell surface
Melanoma cell (Fo-1C cells)	• Increased processing of HLA-I heavy chains
Sarcoma cells	• Increased MHC-I expression
Megakaryocytes (UT7-mpl cells)	• Increased TAP1 and MHC-I expression
Renal Cell Carcinoma	• Increased TAP-1 and β2m expression
Hepatocarcinoma cells	• Increased MHC-I expression
Pancreatic carcinoma cell	• Increased MHC-I expression
Teratoma cells (Tera-2 cells)	• Upregulation of HLA-E and HLA-F alleles
Choriocarcinoma cells (JAR cells)	• Increased TAP1 activity • Upregulation of HLA-E alleles
Colon carcinoma (SW620)	• Increased MHC-I expression
B lymphoblastoid	• Increased MHC-I expression
Monocytic leukemia (j111 cells)	• Increased MHC-I expression

From the data shown in Table 2, it can be concluded that IFN-γ has an impact on the expression of MHC-I molecules and their associated molecules with antigen presentation functions on a wide range of cells. Although this impact was already expected in cells of the immune system (e.g., monocytes, CD4+ T cells) responsible for protecting the body against foreign invaders, other cells not directly involved with this function, such as megakaryocytes, precursors of platelets, also revealed an increase in MHC-I molecule expression. The expression of MHC-I molecules and the MHC-I presentation pathway were also upregulated in β-pancreatic cells in response to IFN-γ. Furthermore, IFN-γ may have an important role by increasing MHC-I

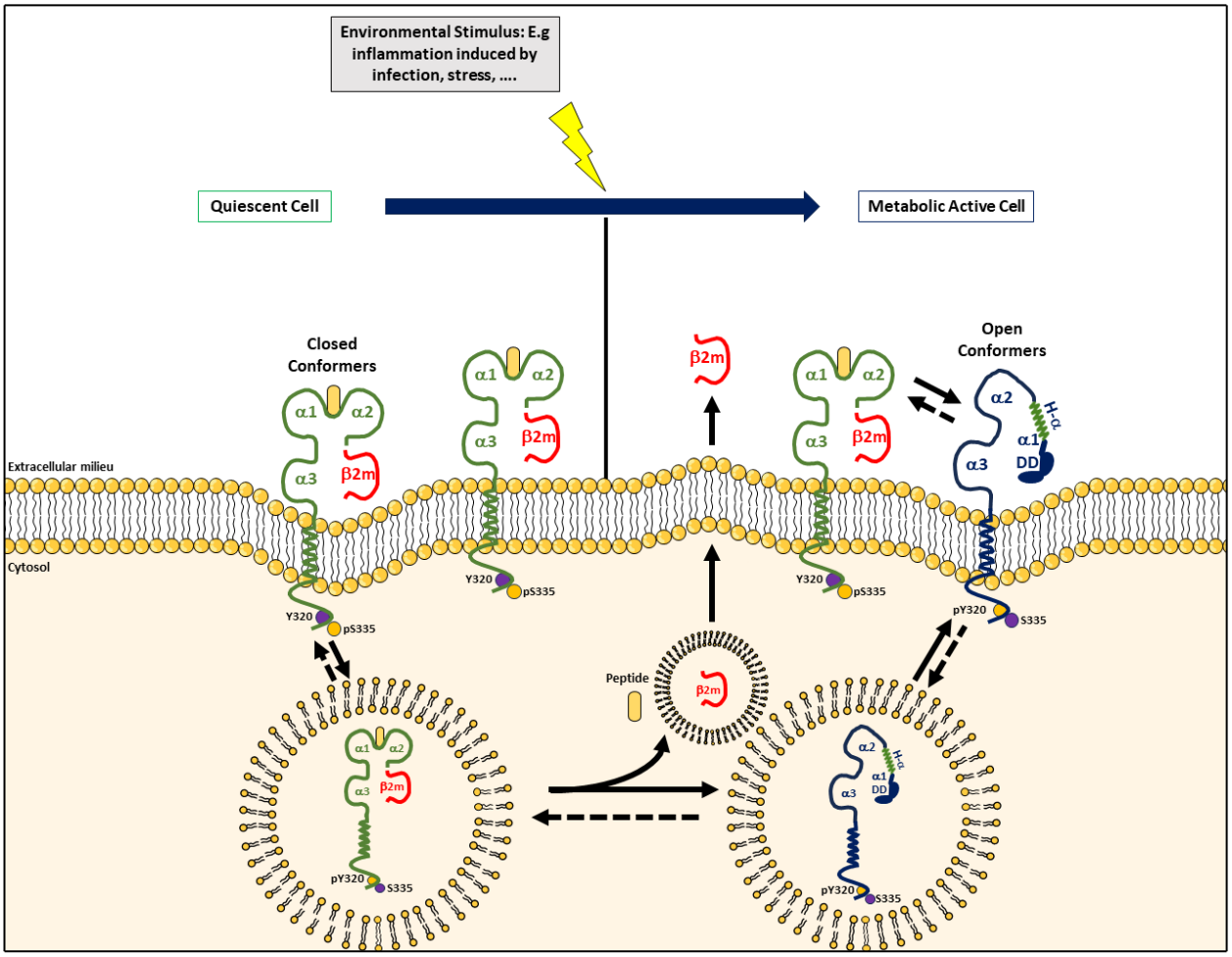
expression in tumorous cells and thus increasing their susceptibility to the cytotoxic effects of CD8+ T cells. Although not shown in Table 2, IFN-γ also influences the expression of MHC-I molecules in cells of the central nervous system.

2.2. MHC-I molecules expressed at the cell surface exist in an equilibrium between β2m-associated and β2m-free forms

Regardless of the type of cell, a fraction of the MHC-I molecules expressed at the cell surface can exist in a physiological equilibrium between β2m-associated heavy chains or closed conformers (the large majority) and β2m-free heavy chains or open conformers. Conditions such as activation, cell growth and inflammation have been proved to be inducers of this conformation (29). As a result, it has been suggested that physiological equilibrium between closed and open MHC-I conformers (Figure 3), and this equilibrium is influenced by nutritional and metabolic demands (30).

Open MHC-I conformers can interact via *cis*-interactions (i.e., interactions that take place in the plasma membrane of the same cell) with themselves, forming homodimers, or with other receptors, forming heterodimers, including the receptors for insulin, epidermal growth factor, glucagon, IL-15, CD8 and others (30). Of note, both single open conformers and homodimers can interact in *cis* and *trans* with a variety of NK receptors both in mice (e.g. Ly49, PirB, etc.) and humans (e.g., KIR and LILR). Moreover, the MHC-I molecules present in the plasma membrane, whether closed or open conformers, can be released into the extracellular environment as "soluble" MHC-I molecules that can be found in a number of biological fluids, including plasma, saliva, urine, sweat, and cerebrospinal fluid (CSF) (30). In this respect, a number of studies in vitro have demonstrated that these soluble MHC-I molecules are immunosuppressor and tolerogenic factors capable of inhibiting NK and CD8+ T cells cytotoxic responses (30).

Figure 3. Model illustrating the balance between MHC-I closed and open conformers in the cell membrane (Adapted from references (30,31))



MHC-I open conformers may result from the endocytosis and reexpression of MHC-I closed conformers. In the resting cell, most MHC-I molecules are in their closed conformer state. Following an initial stimulus, the cell becomes metabolic active and, while some MHC-I molecules are present in their classic form, it initiates the formation of MHC-I open conformers. The MHC-I closed conformer is endocytosed and the tyrosine residue (Y320) located in its the cytoplasmatic tail is phosphorylated by the Src tyrosine kinase Lck (not shown). In the cytosol, it undergoes through a series of events that culminate in the dissociation of the β2m and the bound peptide from the αHC and in the dephosphorylation of the serine residue located in the cytoplasmatic tail of the MHC-I molecule (S335). At this point, the MHC-I open conformer is created and is recycled back to cell membrane, where it is ready to associate with another molecule, either in *cis*- or *trans*-. The detached β2m is secreted into the extracellular milieu. The dashed lines suggest that MHC-I molecules are at equilibrium where MHC-I open conformers can be reexpressed as MHC-I closed conformers by the reverse process.
pS335, Phosphorylated Ser335. pY320, Phosphorylated Tyr320. DD, Disordered Domain

At the RNA level, MHC-I molecules are expressed by a variety of human nucleated cells (see Table 3). However, the level of expression of HLA-I proteins at the plasma membrane differs among different cell types, with monocytes and lymphoid cells presenting the highest levels. MHC-I molecules present at the plasma membrane are not static proteins. Instead, they move freely and are continuously subjected to processes of endocytosis and intracellular trafficking. Thus, during their lifespan at the plasma membrane, MHC-I molecules can be internalized from the cell surface by

endocytosis and either degraded or recycled back to the plasma membrane. Two main paths for the internalization of cell surface receptors were discovered: Clathrin-Mediated Endocytosis, and Clathrin-Independent Endocytosis. MHC-I molecules go through the latter. This endocytic process differs if the cell is or not a professional Antigen Processing Cell (APC) (32).

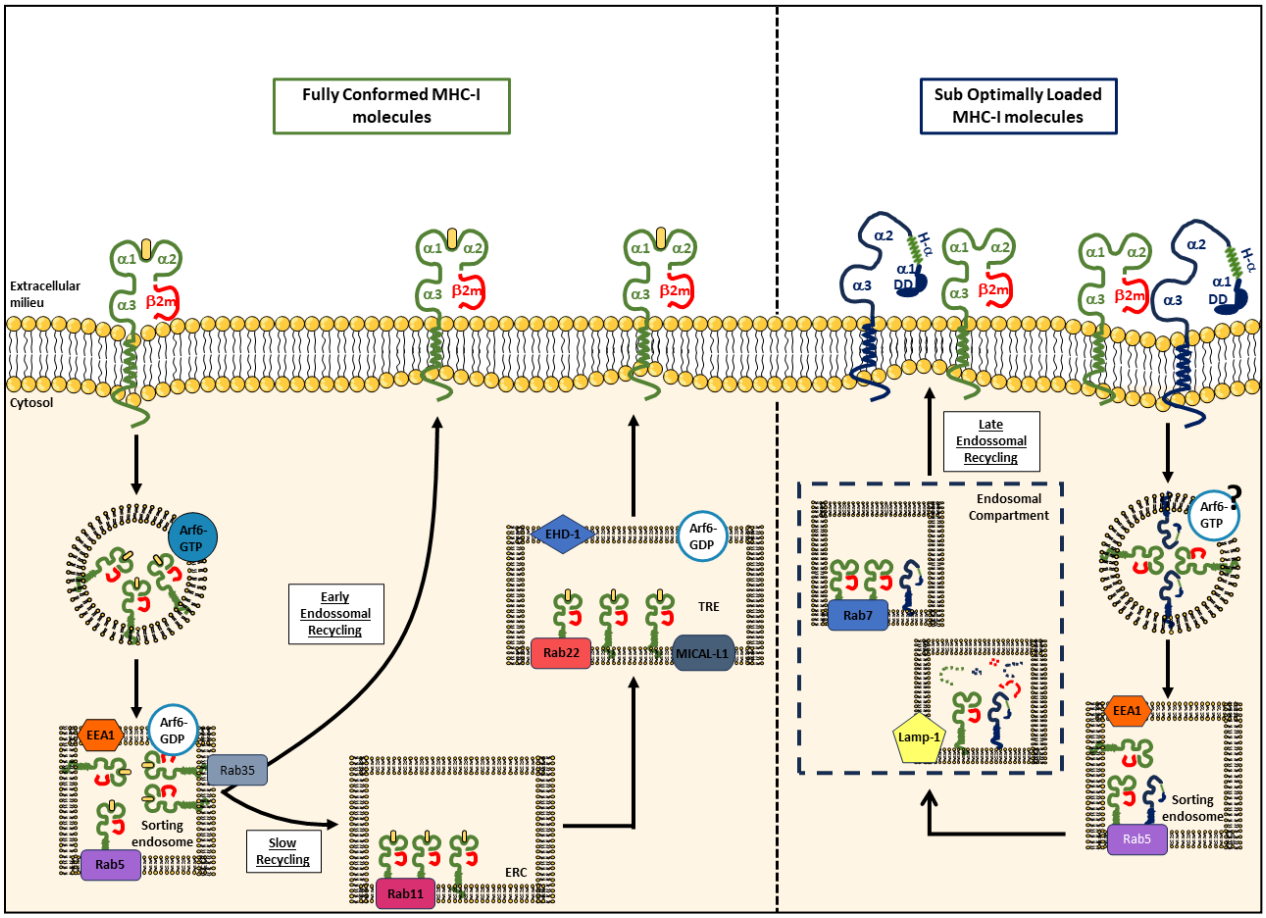
Table 3. RNA expression of HLA class I alleles on different cells and organs*

	HLA class I					
	Classic			**Non-Classic**		
Cells and/or organs	HLA-A	HLA-B	HLA-C	HLA-E	HLA-F	HLA-G
T lymphocytes	++	+++	++	+	+/-	-
B lymphocytes	+	+++	++	+	+/-	-
Dendritic Cells	+	+++	+	+/-	-	-
Monocytes	++	++++	+++	++	+/-	-
Macrophages	+	++	+	+	-	-
Natural Killer cells	+++	+++++	+++	++	+/-	-
Neurons	+/-	+/-	+/-	+/-	-	-
Erythrocytes	+/-	+/-	+/-	+/-	-	-
Hepatocytes	+/-	+	+/-	+/-	-	-
Pancreatic endocrine cells	++	++	+	+/-	-	-
Endothelial cells	++	++	+	++	+/-	-
Trophoblasts	-	-	+	+/-	-	++
Thyroid cells	+	+	+/-	+/-	-	-
Kidney cells	+	+	+	+/-	-	-
Lung cells	+	++	+	+	-	-
Duodenum cells	+/-	-	-	-	-	-

***Adapted from The Human Protein Atlas, https://www.proteinatlas.org/ (accessed, Jul 12, 2023)**
- Do not express; +/- to +++++ increasing levels of expression

In non-professional APCs (see Figure 4), endocytosis occurs primarily via an Arf6-mediated Clathrin-Independent Endocytosis mechanism. After being coated by Arf6-GTP, the endocytic molecule arrives to a sorting endosome, characterized for containing the Early Endosome Antigen 1 (EEA1) and the GTPase Rab5. From here, the destination of the endocytic vesicle depends on the conformation of the MHC-I molecules, that is whether they are fully conformed or not (32).

Figure 4. Schematic representation of MHC-I open and closed conformer trafficking and recycling in non-professional APCs (Adapted from ref. (32))



Proposed model for the trafficking of MHC-I molecules in non-professional APCs. When the MHC-I molecules are fully conformed (left panel), they are endocytosed via the GTPase Arf6 (Arf6-GTP) and transferred to a sorting endosome, containing the Early Endosome Antigen 1 (EEA1) and the GTPase Rab5. From here, they can undergo either early or slow endosomal recycling. During slow recycling, the MHC-I molecule is routed from the sorting endosome to the Endocytic Recycling Compartment (ERC), which includes the GTPase Rab11. Upon arrival to the ERC, it is incorporated into Tubular Recycling Endosomes (TRE) that contain the GTPase Rab22, the EH Domain Containing 1 (EHD-1) protein and the enzyme MICAL-L1. From the TRE, it returns to the plasma membrane. Early endosomal recycling corresponds to a faster process, mediated by the GTPase Rab35, in which MHC-I molecules present in the sorting endosome go directly back to the cell membrane. If the MHC-I molecule is "sub optimally loaded" (right panel), after being in the sorting endosome, they go through degradation compartments, containing the GTPase Rab7. Here, while some "sub optimally loaded" MHC-I molecules are broken down by the Lysosomal associated membrane protein 1 (Lamp-1), others go back to the cell membrane by late endosomal recycling. The presence or absence of the Arf6-GTP during this process is currently unknown.

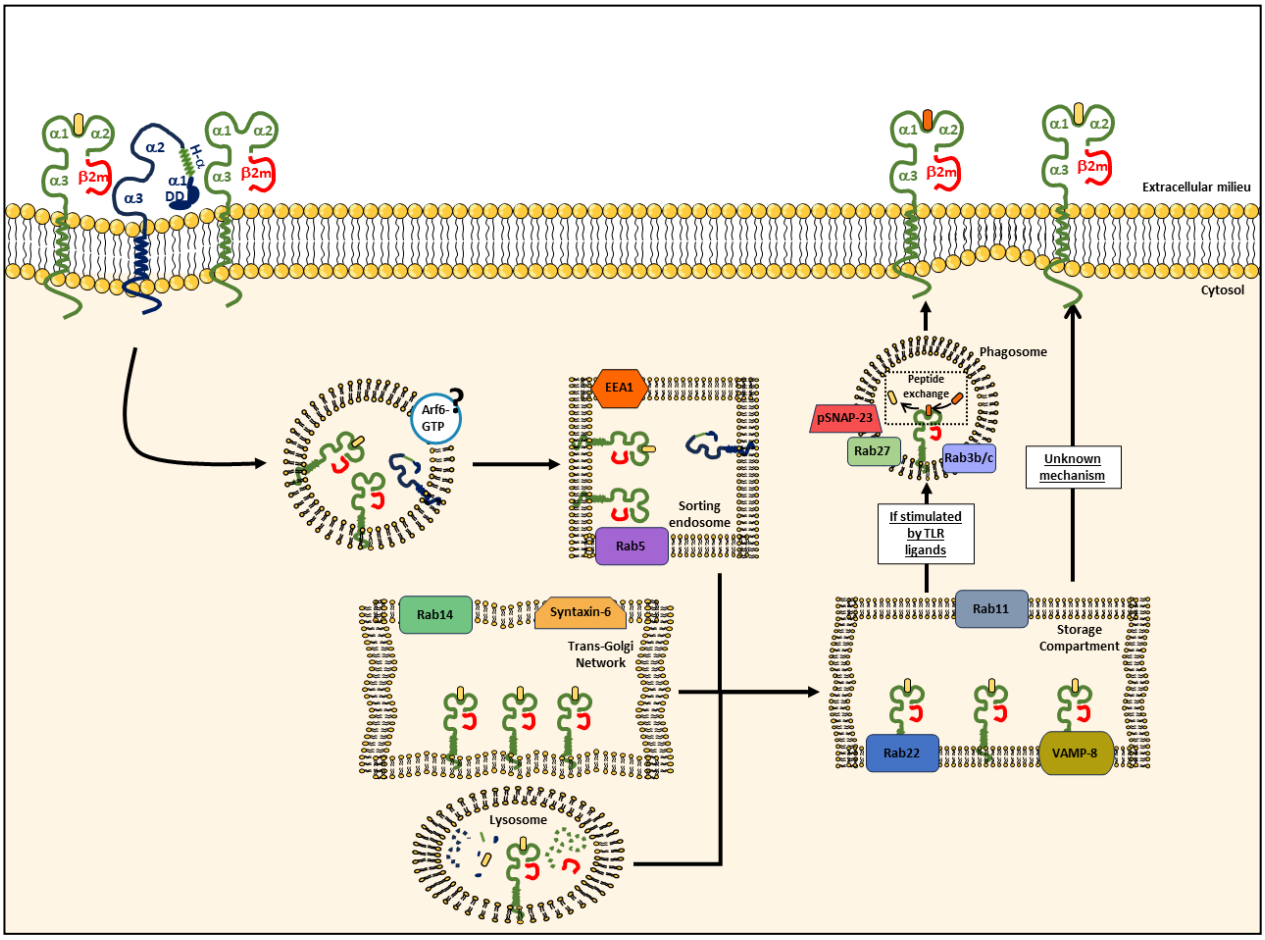
If the MHC-I molecules are fully conformed (Figure 4, left), meaning they are a trimer made up of the αHC, β2m and high affinity peptide, also designated as closed MHC-I conformers (31), they can go under slow recycling or under early endosomal recycling. Slow recycling is the primary method employed by these endocytic vesicles. It involves the transfer of the endocytic vesicle from the sorting endosome to the Endocytic Recycling Compartment (ERC), which includes the GTPase Rab11. Upon arrival to the ERC, it is incorporated into Tubular Recycling Endosomes (TRE) that contain the GTPase Rab22, the EH Domain Containing 1 (EHD-1) protein and

the enzyme MICAL-L1. From the TRE, it returns to the plasma membrane. Early endosomal recycling is a putative faster process in which the MHC-I molecules present in the sorting endosome go back to the cell membrane mediated by the GTPase Rab35. Regardless of the recycling pathway used, Arf6 is present through all this process and is required to ultimately fuse the fully conformed MHC-I molecules with the plasma membrane, thus, returning MHC-I back to its original place (32).

If the MHC-I molecules are "sub optimally loaded" (Figure 4, right), meaning that they are either a single free αHC, also designated as open MHC-I conformers (31), or homodimers of two αHCs, they go under degradation compartments, containing the GTPase Rab7. It is not known if Arf6-GTP is present or absent in this route. Here, some "sub optimally loaded" MHC-I molecules are broken down by the Lysosomal associated membrane protein 1 (Lamp-1) and others go back to the cell membrane by late endosomal recycling (32).

The recycling in professional APCs is less understood (see Figure 5). However, it is believed that endocytosis of MHC-I molecules occurs independently of their conformation, are routed to a "storage compartment", containing the GTPase Rab11 and Rab22 and the Vesicle-Associated Membrane Protein 8 (VAMP-8). This transport can be mediated by the sorting endosome, the Trans-Golgi Network positive for Syntaxin-6 and GTPase Rab14 and even by lysosomes. If stimulated by Toll Like Receptors (TLR) ligands, the "storage compartment" releases fully conformed MHC-I molecules to phagosomes that contain the GTPase Rab3b/c and Rab27 and the phosphorylated Synaptosomal-Associated Protein 23 (pSNAP-23). An environment that is acidic enough to encourage peptide exchange but not so acidic as to degrade MHC-I molecules is assumed to exist during this process. From here, these phagosomes release MHC-I molecules back to the cell surface (32).

Figure 5. Schematic representation of MHC-I open and closed conformer trafficking and recycling in professional APCs (Adapted from ref. (32))



Proposed model for the trafficking of MHC-I molecules in professional APCs. Independently of their conformation and peptide binding, MHC-I molecules are endocytosed to an "storage compartment". At the moment, is thought that lysosomes, the sorting endosome and Trans-Golgi Network containing fully MHC-I conformers and the GTPase Rab14 and Syntaxin-6, may meditate this process. The "storage compartment" contains fully closed MHC-I conformers, the GTPases Rab11, Rab22 and the Vesicle-Associated Membrane Protein 8 (VAMP-8). If stimulated by Toll Like Receptors (TLR) ligands, it releases fully conformed MHC-I molecules to phagosomes that contain the GTPase Rab3b/c and Rab27 as well as the phosphorylated Synaptosomal-Associated Protein 23 (pSNAP-23). During this process some peptide exchange may occur. The MHC-I molecules are then released back to the cell surface by the phagosomes. If not stimulated by TLRs, the "storage compartment" releases fully closed MHC-I molecules to the cell membrane by other currently not understood mechanisms.

Although the mechanisms by which MHC-I molecules present themselves as "open conformers" are still being unveiled, it is now known that the cytoplasmic tail of MHC-I heavy chains includes two conserved motifs that are thought to be biomarkers for the formation of this type of MHC-I molecule. The first motif is constituted by two serine residues (Ser332 and Ser335), in which Ser335 is de-phosphorylated exclusively in open MHC-I conformers (30,33,34), perhaps due to the activity of phosphatases, such as CD45. The second motif is a tyrosine residue (Tyr320 in humans; Tyr321 in mice) that is phosphorylated only in open MHC-I conformers, perhaps due to the activity of the Src tyrosine kinase Lck (31,35).

Interestingly, studies performed in cells transfected with HLA-B27 molecules showed that the presence of Tyr320 in the cytoplasmic tail was associated with the constitutive endocytosis of HLA-B27 molecules to endosomal compartments (36). Indeed, substitution of Tyrosine by a Phenylalanine abrogated endocytosis, pointing to Tyr320 as a key motif regulating MHC-I endocytosis. However, the lack of Tyr320 did not abrogate the generation of open HLA-B27 conformers at the cell surface, suggesting that formation of open MHC-I conformers and endocytosis are, to some extent, separate events (36).

Overall, it can be hypothesized that the non-immunological functions attributed to MHC-I molecules are likely related to the displacement of the closed ↔ open equilibrium to the right, which allows *cis*-interactions with receptors for hormones and growth factors and *trans*-interactions with NK receptors, and to the existence of secreted forms of closed and open conformers in biological fluids, which can interact with their ligands expressed in neighboring cells. In either situation, MHC-I molecules will play a modulator role of the signaling through those receptors.

Chapter 3 – MHC-I MOLECULES AND THE CENTRAL NERVOUS SYSTEM

3.1. Early Studies

As briefly summarized in the previous section, expression of MHC class I molecules was initially described in most nucleated cells, with the exception of cells of the healthy central nervous system (CNS). Indeed, during the 1980s, the investigators could not observe nor induce MHC class I expression in normal neurons beyond doubt, as it was initially described that astrocytes were the only neural cells capable of expressing MHC class I molecules at a basal level and oligodendrocytes could express them after IFN-β or TNF-α treatment (37). Although in the middle eighties Lampson and colleagues showed that IFN-γ increased the expression of MHC-I molecules and β2m, this observation was made using neuronal cell lines and not normal neurons (38,39). It was in 1995, by using cultured rat hippocampal neurons, that Neumann and colleagues working in the group of Hartmut Wekerle at the Department of Neuroimmunology of the Max Planck Institute for Psychiatry in Germany, demonstrated that the MHC class I gene transcription was very uncommon in normal neurons with spontaneous action potentials (40). However, in electrically silent neurons, transcription was detected, with a more precise regulation of expression of β2m compared to class I heavy chain molecules. Noteworthy, only electrically silent neurons treated with IFN-γ had surface expression of class I molecules (40). Electrically silent neurons are found in silent synapses, which are undeveloped connections between neurons that lie dormant until they are called upon to help in the formation of new memories, and they are abundant in the adult brain (41). Following studies by the same group showed similar results when TNF-α was used (42).

From these investigations, the authors concluded that the transcription of both MHC class I heavy chain and β2-microglobulin genes could be achieved in neurons; consequently, neurons can display MHC class I molecules on their surfaces, equipping them with the fundamental elements needed to engage with CD8+ cytotoxic T cells (40,42). The practical implications of these early findings were

related to the immune surveillance of viral infections of the CNS, which led Neumann and colleagues to suggest the capacity of cytotoxic T lymphocytes (CTLs) with a CD8+ phenotype to identify and destroy brain cells that express MHC-class I molecules (43), a view that it is still dominant in the neuroimmunology field.

3.2. Breakthrough Studies

Following these initial studies, others expanded and further developed these findings. A series of seminal studies carried out in mice models by the group of Carla Shatz brought about a radical change in the way neuroscientists and immunologists looked at MHC class I molecules. These studies demonstrated for the first time that MHC-I molecules were not only triggers and targets of an immune response, but they also played a critical role in CNS homeostasis (44,45).

In the seminal study of Corriveau et al (44), they analyzed feline genes expressed during the development of the Lateral Geniculate Nucleus as eye-specific layers form, along with genes influenced by spontaneously occurring action potentials in the retinogeniculate pathway, using screens for differential mRNA display. Surprisingly, MHC-I was identified in the screen and was found to be expressed in normal conditions in subsets of neurons in vivo. In the same study, CNS neurons were found to express both β2m and CD3ζ, a component of the T cell receptor (TCR/CD3) complex of T cells. Interestingly, while MHC-I class mRNA levels were downregulated by inhibiting spontaneously evoked or visually driven activity in prenatal and postnatal Lateral Geniculate Nucleus using the sodium blocker channel Tetrodotoxin, their expression was upregulated in a mice seizure model. Ultimately, these results showed a dynamic regulation of MHC-I mRNA levels during development, both in the visual system and in the hippocampus. In the following seminal study of Huh et al (45), mice genetically deficient for cell surface MHC-I molecules were studied. The results demonstrated that mice deficient in MHC-I molecules displayed aberrant neuronal connections, pointing to a direct neuronal role for MHC-I signaling through an unidentified CNS-specific or a known immune receptor. In the same study (45), it was observed a differential MHC-I subclass expression in distinct neurons of the brain, which may be related to these molecules having different brain functions.

These two studies were followed by others that convincingly showed that MHC-I expression takes place in the CNS, and had an unexpected impact in brain development, neuronal remodeling and synaptic adaptability (46–48). These findings lay the groundwork for further investigation into the role of MHC-I molecules in the pathology of neurological diseases (49).

3.3. Confirmation Studies

Following the seminal studies from the group of Carla Shatz, neuroscience researchers started to conduct investigations to confirm and extend these original findings. These studies, carried out majoritarily in manipulated animal models, are described below.

Among the different cell component of the CNS, neurons are the main cell type expressing MHC-I molecules (50,51), and one prominent aspect observed in various studies is that MHC-I molecules were mostly present in the developing CNS during mice gestation, including neural stem cells (50,52). These findings were suggestive of brain MHC-I proteins exerting a non-immunological function during the earliest stages of life. However, MHC-I expression was also observed in the brainstem and in the brain of adult mice (53,54), suggesting the involvement of MHC-I molecules in the refinement of synapses in response to the occurrence of neural events, such as aging. These studies led to the conclusion that MHC-I molecules may participate in various stages of neural development. Indeed, other groups showed that MHC-I molecules were intertwined to the processes of neural circuitry regulation and synaptic plasticity, where synapses adapt according to intrinsic or extrinsic stimulus, with MHC-I expression in mice brain during development reaching its peak during synaptogenesis (54). Interestingly, MHC-I proteins were detected close to the synaptic cleft, being related to synaptic structures such as axons and dendrites and to the elimination of synapses during normal gestation (50,54–56).

On the other hand, during eye formation and eye interventional procedures in mice, absence of MHC-I expression was correlated with an abnormal eye dominance plasticity, meaning the normal function of the brain to adapt visual inputs from one eye compared to another was compromised to some extent (57,58). One explanation for these phenomena can be seen when considering that cortical neurons of mice

lacking β2m mRNA expression displayed a reduction in the number of surface MHC-I proteins and an increased density in excitatory glutamate synapse, although there was a smaller increase in inhibitory Gamma-Aminobutyric Acid synapses as well (55). Moreover, neurons that have a physiological lack of β2m expression, such as those innervating ocular muscles, were highly electrically active (53). Meanwhile, cortical neurons of mice overexpressing MHC-I molecules had a lower density of glutamate synapses and, overall, a reduced excitatory state (55). From these studies, it is likely to think that an increase in MHC-I expression caused by stress or inflammatory cytokines inhibits the formation of excitatory synapses and limits the density of synapses in general. Indeed, a number of subsequent studies, have confirmed these results (55,56).

Although the importance of the previous studies on MHC-I molecules during development are undeniable, they are limited by the fact that they were performed on animal models. Thus, this may bring the question of whether MHC-I molecules may also have an impact on human brain formation. Remarkably, it has been shown that, throughout the 31-to-33-week gestation period, the human hippocampus undergoes a gradual maturation process in which there is an increase in the levels of the MHC-I αHC and, to a lesser extent, in the content of β2m. Furthermore, this increase in the expression of MHC-I molecules was only present on human hippocampal neurons, mainly in glutaminergic neurons, and not on other immune brain cells, and it disappeared during adulthood (59). Similarly, the presence of MHC-I protein expression has been reported in other human brain areas, such as the cerebellum and brainstem, where they were, once again, most prevalent during the gestation interval (60,61). The fact that MHC-I proteins were located preferably close to the synaptic cleft in fetal human brains (61) points to a possible regulation role in synaptic formation, namely in those that have an excitatory nature.

Importantly, the abnormal presence of MHC-I molecules in the neural circuitry during its formation can have important neurodevelopmental implications. It was reported that mice overexpressing the H-2D^{d} allele showed morphological changes in their hippocampus and were less capable to recover hippocampal-dependent spatial tasks from lesions in the pathways of this region due, in part, to a disturbance in the sprouting of compensatory synapses (51). In addition, a study reported that mice lacking the H-2K^{b} and H-2D^{b} alleles had an abnormal increase in the density of

neuromuscular junctions (NMJ), where neurons come into touch with muscle fibers (62), results that resemble the ones of Marin *et al* (57), Datwani *et al* (58) and Tetruashvily *et al* (63). A similar pattern was seen in the diaphragms and NMJ of mice lacking β2m and TAP. During the second postnatal week, muscle innervation was abnormally increased, resulting in synaptic strengthening. In contrast, mice overexpressing H-2D^b had their motor innervation significantly reduced (63). These data suggest that MHC-I molecules may be involved in the removal of excessive synaptic activity in this area, contributing to the normal motor function during development (64) and recovery after lesion (62). Indeed, this reasoning can be better understood in the context of nerve lesions followed by regeneration.

A brief summary of the findings described in this section can be found in annex I.

Chapter 4 – MHC-I MOLECULES, AGING AND THE PROCESSES OF NEURODEGENERATION AND NEUROREGENERATION

Aging is a complex physiological process that involves several structural changes. The nervous system is one of the main components affected while neurons become less capable of auto repairing and more susceptible to the processes of neuroinflammation. Analyses of the NMJ and spinal cord of aged mice showed an increase in pro-inflammatory cytokines and in the expression of MHC-I molecules (63,65) that, ultimately, may have an important correlation in maintaining the neuronal structural architecture and complexity.

For instance, aged mice lacking expression of MHC-I exhibited altered hippocampal neurons (66). Both the apical dendrite length and the distance from the dendrite to the soma were significantly shorter than normal. Additionally, the shape of the spine was affected with a predominant increase in thin spines and decrease in stubby spines. Certainly, these findings had implications in the synaptic function of these neurons as there was an increase in the number of total synapses and in the expression of synaptic proteins, such as the N-Methyl-D-Aspartate receptor subtype 2B subunit of the N-Methyl-D-Aspartate receptor. Accordingly, MHC-I molecules may control neuron remodeling and integrity. In the context of aging, these processes become gradually more complex and the amount of MHC-I molecules required for their correct regulation must increase, which may explain their reported increased expression with aging (63,65,67,68). Accordingly, it has been reported that MHC-I molecules may contribute to aging-related synapse loss in mature muscle fibers as muscles of aged mice lacking β2m and TAP showed less motor denervation in comparison with their aged wild-type (WT) counterpart (63).

In a recent study where mice brains were analyzed, it was revealed that MHC-I molecules were mostly expressed in the microglia and that their level of expression increased with age (67). Similar findings have been observed when post-mortem human brains were examined (67). Most strikingly, studies on aging mouse brain and older human brains unveiled that while microglia expressed MHC-I-binding leukocyte immunoglobulin-like receptors (LILRs) and paired immunoglobulin-like

type 2 receptor families, astrocytes and neurons did not (67). Thus, these receptors have the potential to facilitate cell-autonomous MHC-I signaling and are expected to increase with age in both mice and humans. Interestingly, increased microglial MHC-I, LILR, and paired immunoglobulin-like receptor expression was observed in various mouse models of Alzheimer's disease (67).

With the confirmation that MHC-I molecules are present in the CNS during development, their changes with aging and their possible role in neuronal degeneration and/or regeneration takes central stage. Although most studies dug into this question in relation to MHC-I molecules in their classic closed conformer form, others delved into their alternative forms. As mentioned above, under certain circumstances the αHC-β2m-peptide trimeric conventional structure of MHC-I molecules, the closed conformers, may dissociate generating free αHCs, the open conformers. As a direct consequence, β2m is released into the extracellular milieu as soluble β2m. Not only these forms may be linked to the process of aging and to the development of neurodegenerative conditions, namely Alzheimer's disease (AD) and Parkinson's disease (PD), but they could also be powerful therapeutic targets. In this regard, it is important to highlight the fact that neurodegenerative disorders can be caused by the low-chronic neuroinflammation that is associated with aging, which results in the production of numerous cytokines, including IFN-γ and TNF-α, as mentioned above.

4.1. Animal Models of Neurodegeneration

Alzheimer's disease is a neurodegenerative disorder associated with the deposition of toxic $A\beta_{42}$ protein aggregates and the formation of plaques. These plaques tend to accumulate in the CNS parenchyma, and they initially activate the innate immune system. This response is mediated by microglia and astrocytes that try to clear these protein aggregates. However, since the rate of the production of Aβ protein aggregates far surpasses the rate to which the immune system clears them, it induces a chronic neuroinflammation that results in the loss of neurons, especially in the hypothalamus, and leads to dementia (69). It has been observed that brains of AD patients contained peripheral T cells, suggesting that the chronic neuroinflammation process promotes some degree of T cell infiltration through the Blood Brain Barrier

(BBB). The main function of the BBB is to control and block the passage of toxic content into the brain and is composed by astrocytes, endothelium cells, among other components (70). For instance, in an study conducted with Human Microvascular Endothelial Cells, it was found that, after injecting mouse brains with $A\beta_{1-42}$ protein, there was an increase of the number of endothelial mice brain cells expressing MHC-I molecules (71). Additional studies led the investigators to conclude that Aβ proteins could be captured and incorporated by microglia, which then release TNF-α, leading to the expression of MHC-I molecules on the brain endothelium which allowed T cells to cross the BBB and migrate into the brain (71). In this model, it was reported that anti-MHC-I antibodies blocked the migration and adhesion of T cells. Also, the use of anti-TNF-α antibodies, instead of anti-MHC-I antibodies, showed a decrease in T cell migration and adhesion, which pointed to the possibility that the transendothelial migration of T cells is additionally influenced by TNF-α. These results are consistent with the presence of activated T cells in peripheral blood and the choroid plexus of the brain of Alzheimer's patients, which subsequently move into the brain parenchyma (72). Hence, it is possible that the migration of peripheral immune cells to the brain may occur due to specific MHC-I interactions with endothelial receptors. In fact, one study found that MHC-I molecules were capable of interacting in *cis* with integrin β4, an important molecule for cell adhesion and migration between the cytoskeleton and the extracellular matrix, while also being capable of influencing cell physiology (73). Although present in many tissues, integrin β4 was recently found to be a component of the BBB (74). Both molecules were colocalized on the basal membrane of human endothelial cells and transfection of these cells with integrin β4 Small Interfering RNA was accompanied by a reduction in HLA-induced cell proliferation (73). Likewise, changes on MHC-I molecules also interfered with the actions of integrin β4. More specifically, deletion of the cytoplasmatic tail of the HLA-A2 molecules compromised the formation of complexes with integrin β4 and blocked endothelial cell proliferation. Interestingly, all these disrupting effects were absent in the HLA-A2 molecules that had only their extracellular domain deleted, suggesting that the cytoplasmatic tail of MHC-I molecules is crucial for the correct function of integrin β4. Moreover, endothelial cell transfection with both integrin β4 and MHC-I heavy chain and β2 Small Interfering RNA also impaired cell migration across an artificial

wound (73). When all these findings are considered, it can be proposed that *cis*-associations of MHC-I molecules with integrin β4 could regulate the migration of different immune cells into the brain. So, in the context of neurodegeneration, the buildup of neuroinflammatory debris could compromise this association, leading to an aberrant BBB and decreasing neuron regeneration and survival.

Regarding other functions of MHC-I molecules in AD pathogenesis, it was recently reported that they could be related to the presence of the ApoE gene (75), one of the most linked genes with this condition. In the pathophysiology of AD, there is a pronounced reduction in selective acetylcholinergic neuron populations, namely those in the hippocampus. Although, the processes that determine which neurons are eliminated remain currently unknown, this study proposed that ApoE is one possible culprit. By using humanized ApoE3 and ApoE4 Knock-In mice models, high levels of ApoE expression were present in the hippocampus, being associated with several neural stress pathways, such as neurodegeneration, unfolded protein response and immune response (75). Within the hippocampus, other significant changes were present such as a marked reduction in synaptic content and in the total hippocampal volume. Curiously, only specific hippocampal neurons displayed high content of ApoE expression and, therefore, were more susceptible to neurodegeneration. Hypothetically, MHC-I molecules could signal the selective neuronal elimination since ApoE expression was positively correlated with immune response, possibly due to induction of neuroinflammation. In fact, ApoE expression closely tracked MHC-I gene expression in ApoE-knock-in mice neurons. When ApoE expression was abolished, not only ApoE and MHC-I protein content declined, but it also reverted the synaptic loss and hippocampal shrinkage. In another finding that corroborates the role of MHC-I molecules in AD, both absence of β2m or MHC-I expression in mice neurons decreased tau protein mislocalization, which is another AD protein misfolding pathway where the phosphorylated tau protein becomes entangled, leading to neuronal degeneration (69).

Parkinson's Disease is another prevalent neurodegenerative condition. Various factors, including chronic stress, cause a loss of dopaminergic neurons of the substantia nigra and loss of norepinephrinergic neurons of the locus ceruleus, which leads to cognitive impairment and impaired motor movement (76). The processes

that lead to the death of these neurons are currently unknown. However, there are various studies that try to explain this process. The main hypotheses include the presence of oxidative stress induced by mitochondrial dysfunction in dopaminergic neurons; neuronal inflammation in the substantia nigra and genetic mutations that affect the PTEN-induced kinase 1, protein deglycase DJ-1 and leucine rich repeat kinase 2 genes (77).

In this respect, Wang et al. examined how stress impacted on MHC-I expression in dopaminergic neurons and how this can be related to the development of PD, by using an animal model of PD (78). In this study, it was observed that injection of brains of mice–with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1-methyl-4-pheylpyridinium, substances known to induce neuron inflammation and cause PD in animal models, increased the loss of dopaminergic neurons, the expression of dopaminergic MHC-I^{+} neurons and CD8+ T cell infiltration (78). Interestingly, a study by David Sulzer's group analyzing postmortem human brain samples, showed that MHC-I was expressed by human catecholaminergic substantia nigra and locus coeruleus neurons, and that human stem cell-derived dopamine neurons could also induce the expression of this molecules (79). The authors suggested that microglial activation, secretion of IFN-γ, the release of α-synuclein, and high cytosolic dopamine, may be the cause of neuronal MHC-I expression and antigen presentation in catecholamine neurons, which in the presence of the right antigen and CTLs, could contribute to neuronal death in diseases where there is significant CNS inflammation (80).

Besides IFN-γ and TNF-α, IFN-β is another pro-inflammatory cytokine that may influence MHC-I molecule expression and give a more meaningful insight into how this mechanism is regulated on neurons. In the work of Ignarro et al. on the effect of IFN-β in human astrocytes (81), it was shown that, after treatment with IFN-β, there was an increase in the expression of the β2-microglobulin light chain. Also, it was reported an increase in their granulation as well as in the expression of Vimentin and Glial Fibrillary Acidic Protein. Both proteins are crucial for the construction of filaments on developing astrocytes and their synthesis is upregulated during an immune response, being used as markers for the activation of astrocytes after lesion

on neurons. This correlates with the argument that, following IFN-β secretion during inflammation there is an upregulation in the activity of astrocytes.

Concomitant with these results, the presence of IFN-β was associated with an increase in the expression in other pro-inflammatory cytokines such as IL-1β and IL-6. While this may suggest that IFN-β engages in promoting pro-inflammatory conditions that may be harmful to the astrocytes, this cytokine did not induce inflammatory changes when injected into rodent adrenal medulla cells. In fact, when astrocytes were exposed to IFN-β, the expression of the glutamate transporters Glutamate Transporter-1 and Glutamate Aspartate Transporter was upregulated. Glutamate is an aminoacid used as an excitatory neurotransmitter and cannot be degraded in the extracellular environment. Thus, to prevent accumulation of glutamate in the synaptic cleft and potentially neurodegeneration, these transporters promote the uptake of the aminoacid into glial cells. In this regard, it is important to note that the synaptic vesicles present in these clefts express MHC-I molecules in the plasma membrane throughout neurodevelopment (54). Taking all this into account, it can be suggested that IFN-β may have neuroprotective properties.

A brief summary of the findings described in this section can be found in annex II.

4.2. Human Neurodegenerative Disorders: Alzheimer's and Parkinson's

4.2.1. HLA-I molecules and Alzheimer’s disease

When studying individuals with Alzheimer's disease, researchers discovered that certain HLA-A, HLA-B and HLA-C alleles were more prevalent in the presence of this disease condition. In relation to HLA-A alleles, when individuals with AD with late onset were compared to those with early onset, there was a substantial increase in the frequency of the HLA-A1 allele in the former group, especially in patients with AD onset greater than 74 years old (82). This might imply that this allele has a “protective” effect on the symptomatic emergence of the illness as their carriers were more likely to develop symptoms later in life. HLA-A2 is another key allele for the relationship between AD and HLA class I. Although initial studies found no link between HLA-A2 and AD (83), more recent research suggests that this is not the case

and that there is some association (84). It was discovered that the HLA-A2 allele was linked to an earlier onset of AD (85) and that there may exist a familiar component when it comes to this allele. Individuals with familiar AD had a higher prevalence of this allele when compared to patients with sporadic AD (86). Also, the HLA-A2 allele was linked to the atrophy of the right hippocampus (87). Thus, the effect that the HLA-A2 allele may have on modulating brain structures could be interpreted as an indication to the hypothesis that individuals carrying certain HLA alleles may be predisposed to neurodegeneration. In support of this assumption is a recent study showing that changes in a nucleotide, known as Single Nucleotide Polymorphisms, of the HLA-A gene were associated with atrophy of different parts of the brain. The Single Nucleotide Polymorphism rs76475517, which only exists in Caucasians with the HLA-A23 and HLA-A24 alleles, was associated with atrophy of the right amygdala and the left hippocampus in individuals either with AD or Mild Cognitive Impairment (87). Intriguingly, a recent study in a Portuguese cohort of elderly subjects (EBIcohort, mean age, 81 years old), some of them with AD, showed a close relationship between the presence of the HLA-A23 and HLA-A24 alleles, dementia and the levels of soluble HLA-I molecules in plasma (88).

In relation to the HLA-B and HLA-C alleles, a study found that the frequency of HLA-B15 was higher in patients with presenile and senile dementia of AD, which may pinpoint this allele as a risk factor for AD (89). The presence of another HLA-B allele, HLA-B7, was also associated with an early start of AD as well as an increased severity of the illness (90,91). Furthermore, there was a demonstrated higher frequency of the HLA-Cw*0702 alleles in AD patients (91). Also, a linkage disequilibrium was confirmed between HLA-B7 and HLA-C*0702, indicating a non-random relationship between them (91). The frequency of AD was also higher among homozygotes carrying the HLA-B7 and the HLA-Cw*0702 alleles. As noted by Lehman et al. (91), homozygosity for these alleles was associated with low Natural Killer cell activity and with greater susceptibility to infections. So, it can be suggested that homozygosity in these carriers can decrease Natural Killer cell activity and thus increase vulnerability to AD as the immune system is dysregulated and more vulnerable to processes of inflammation. Interestingly, a previous study made by Lehman *et al* (92), found conflicting evidence for the association of the HLA-B7 allele with the late onset AD and concluded that there was no significant correlation. More

recently, Esgalhado *et al* showed that the HLA-B8 allele was more frequent in the elderly volunteers of the EBIcohort with severe cognitive impairment when compared to elderly with no cognitive impairment (93).

It is worth mentioning a genotyping and CSF analysis made from studying two major datasets of patients with AD (94). In these cases, the A*03:01, B*07:02, DRB1*15:01, DQA1*01:02, DQB1*06:02 haplotype was associated with higher β-amyloid levels in CSF. Also, this same haplotype was significantly increased in men with AD. This finding is relevant because it may indicate that male individuals carrying this haplotype may be more prone to neurodegeneration. In contrast, the frequency of the A*02:01, B*13:02, DRB1*07:01, DQA1*02:01, DQB1*02:02 haplotype was decreased in patients with AD, namely in females, which points to a possible protective effect against AD in females. Once more, the influence that HLA class I alleles have on AD is evident and, even more importantly, they may have a relation with sex and the formation of β-amyloid aggregates and plaques.

Under normal conditions, the immune system is trained to differentiate between self and non-self-antigens and to accept self-antigens in order to not develop autoimmune conditions. A "high self-antigen load" is a condition in which the immune system is exposed to a significant number of self-antigens, which may lead to the production of autoantibodies and to the dysregulation of the immune response. As previously mentioned, AD can be a result of a chronic auto-inflammation that leads to neuron death. If there exists a stimulus that perpetuates and precipitates this state of inflammation (e.g., high-self antigen load in germline mutations), neurodegeneration, and consequently AD, may occur earlier. In fact, a study found that there was a significant increase in the self-antigen load in patients with early-onset AD and the disease was more severe when compared to late-onset AD patients (85). Although not suggested in this study, it can be speculated that, as the alleles mentioned are associated with an early onset of AD, they may influence the increase in self-antigen load which may cause this condition. With these associations, it can be hypothesized that the presence of all these alleles in the surge of AD may result in an increase in the expression of MHC-I molecules on the surface of cells present in the brain parenchyma. In support of this assumption is a study showing that the capillary profile, as well as the grey and white matter microglia, in patients with this condition exhibited enhanced MHC-I molecule staining (95).

4.2.2. HLA-I molecules and Parkinson's disease

Studies on the association between HLA Class I alleles with Parkinson's Disease are scarce and conflicting. While some studies showed that HLA class I alleles had no association with this disease (96), others concluded that there may be some degree of connection (97–99).

When studying a small sample of an American-Jewish patients with PD, Elizan *et al* (97), found that the HLA-B14 allele was increased in this group. In another study with a larger sample of patients of European descent with diagnosed PD, Wissemann *et al* (98) observed that the frequency of the HLA-B*07:02 and HLA-C*07:02 alleles was higher among patients with PD and the frequency of the HLA-B*40:01 and HLA-C*03:04 alleles was lower. Thus, it seems that, while the presence of the HLA-B*40:01 and HLA-C*03:04 alleles could confer protection against PD, the presence of the HLA-B*07:02 and HLA-C*07:02 alleles may be harmful. Interestingly, as mentioned previously, these latter alleles were more frequent in patients with AD. Therefore, it can be suggested that the carriers of these alleles are more likely to develop some type of neurodegeneration.

Finally, it is worth noting that in the same study of elderly volunteers of the EBIcohort referred above, which included PD patients, showed that the large majority of these patients suffered from dementia and carried the HLA-A23 and HLA-A24 alleles, pointing to these alleles as potentially harmful for developing neurodegeneration (88).

Even though these results are encouraging, many of these conclusions cannot be extrapolated to the general population and are conflicting (92,100). These discrepancies may be due to the variation in sample size and even differences between populations, as some HLA class I alleles are more predominant in certain ethnic groups (82–84,89). Therefore, a better understanding of the correlations between HLA-I alleles and neurodegenerative disorders requires increasing the size of the samples under study and having similar inclusive criteria, so the results become easier to compare and interpretate. Moreover, a better understanding of the presence of HLA-I ligands (e.g., LILR, KIR, etc.) in these subjects and the existence of possible *cis*-associations may help in our comprehension of these phenomena.

4.3. β2-microglobulin and neurodegenerative conditions

There are currently many studies that dwell into the conditions that promote β2m dissociation from the αHC and the subsequent release into the extracellular environment (see Section 2.2). Although at this time of writing the fate of the unbound peptide remains a mystery, some studies speculate that the release of β2m may be associated with cognitive declining due to aging or neurodegenerative disorders.

In relation to aging, when compared to healthy younger individuals, β2m plasma concentration was found to be higher among older healthy patients and even higher in individuals with dementia (101,102). Similarly, plasma β2m content was increased in aged unpaired mice (102). Curiously, young mice at 3 months exposed to aged mice blood at 18 months showed an increase in the β2m plasma levels. This finding was also seen in heterochronic parabiotic mice, which corresponds to the fusion of two mice with different ages that have a shared circulatory system (102). This raised the question of whether β2m functions as a factor that accumulates with aging and if it has any cognitive implications. To solve this question, mice were injected intraorbitally with β2m at 3 months of age and were tested in different cognitive environments. Impressively, mice injected with β2m displayed worse cognitive scores and even had a significant decrease in hippocampal neurogenesis. In a surprising turn of events, older mice with suppressed β2m expression performed better in cognitive tests than their aged unpaired counterpart. The negative effects of β2m on cognitive function were reversed after a lengthy period of recovery, suggesting that these effects do not exist in the long-term (102). These important results point to β2m being a possible novel biomarker for aging and cognitive impairment.

Regarding neurodegenerative diseases, initial studies in rodents reported high levels of both MHC class I heavy chain and β2m mRNAs in motoneurons and nigral dopaminergic neurons, which is one the first targets for the development of Parkinson's disease, in the brainstem of the adult rat (53). Subsequent studies in humans showed that the levels of soluble β2m were increased in the CSF and brain parenchyma of Parkinson's disease patients (103,104). Similar results were reported

when the levels of soluble β2m were studied in plasma samples from Alzheimer's patients when compared to healthy and Mild Cognitive Impairment subjects (105). A link between high levels of soluble β2m and brain disfunction was shown in two different studies using animal models (106,107).

In the first study, using a mice model of Alzheimer's disease, β2m was shown to co-aggregate with β-amyloid and contribute to amyloid pathology (106). Importantly, in this mice model of AD, the levels of β2m increased with age and injections of β2m increased not only amyloid hippocampal deposition, but also the formation of dystrophic neurites, which are abnormal neuronal processes. Moreover, β2m protein expression was found to be localized majoritarily in the microglia, and not in astrocytes or neurons, of the hippocampus. These findings are interesting because the microglia are the innate immune cells of the central nervous system. Thus, perhaps the β-amyloid toxicity driven by β2m accumulation could be caused by a dysregulation of microglia function. Microglia activation will hasten the dissociation of the closed MHC-I conformers generating open MHC-I conformers and free β2m, which is released into the microglia microenvironment. The possible role played by the open MHC-I conformers on microglia is presently unknown. Noteworthy, additional studies using transgenic mice lacking expression of MHC-I molecules showed that injection of β2m led to β-amyloid deposition. In fact, CSF and blood plasma with high levels of β2m protein has been associated with patients with AD (108–110) and the possible underlying mechanisms can be found on the Chinese Alzheimer's Biomarker and Lifestyle study (111). This study analyzed a cohort comprised by cognitively healthy adults that was then divided into those with negative biomarkers for Aβ amyloid plaques but with abnormal markers of neurodegeneration and those with positive biomarkers for Aβ. According to this study, increased plasma β2m content was linked with individuals that were more prone to the deposition of Aβ plaques in the brain, as seen by the low levels of $A\beta_{1-42}$ in the CSF, and associated with worse cognitive test scores. Moreover, statistical analysis revealed that $A\beta_{1-42}$ mediated the correlation between β2m levels and cognitive scores (111). These results, together with clinical data, bring about the hypothesis that an increase in the β2m levels may contribute to the misfolding and formation of β-amyloid plaques or the way around. Indeed, the results of two recent

and separate studies strongly suggest that β-amyloid induces the dissociation of cell surface closed conformers into membrane-anchored αHC and β2m, which is likely secreted into the extracellular milieu (112). Then, β2m coaggregates with β-amyloid and contributes to amyloid pathology and cognitive deficits in Alzheimer's disease model mice (106).

In the second study, using a rat model of stroke, which can be another risk factor for cognitive impairment, Chen *et al.* (107) found that the levels of β2m in the serum, CSF and brain tissue of the rat increased following ischemic-hypoxic brain injury, where they had their middle cerebral artery occluded to mimic a stroke. This rise in β2m content peaked at 3 hours following brain damage and returned to normal levels in CSF and serum at 3 and 14 days, respectively. When these animals were treated with an interference RNA that blocked β2m translation, the β2m content in serum, CSF and brain tissue decreased, as expected, and was accompanied by a reduction in the infarct volume and cognitive deficit, as well as a decrease in glial cell activation and in the activation of the pro-mediator of inflammation after stroke, NLR Family Pyrin domain containing 3. From this study, it can be speculated that, after stroke, an increase in the β2m content promotes an inflammatory response that leads to neuron damage and impairs cognitive function and glial activation. When β2m is inhibited, this neuroinflammatory state is reversed which could lead to a lesser degree of cognitive side-effects. Possibly, in this context, β2m could be used to assess the degree and prognosis of brain injury. As it has been shown, urine and plasma β2m content was increased following brain lesion and positively correlated with its severity (113). Interestingly, therapy with hyperbaric oxygen reduced the plasma levels of β2m (113). Thus, the increase in β2m expression induced by neuronal damage and death during stroke could be used as a biomarker in order to detect early brain injury.

A brief summary of the findings described in this section can be found in annex III.

4.4. MHC-I molecules and neuroregeneration

The aforementioned results raised the fundamental question of whether MHC-I molecules could be involved in neuroregenerative processes after neuronal damage. In this respect, a number of studies have addressed this question by using WT, knock-out mice for β2m and MHC-I molecules, and β2m and TAP1 deficient mice. While knock-out mice completely lack the knocked-out proteins, deficient mice express low levels of the proteins. A study where the sciatic nerve of β2m knock-out mice was resected, showed an aberrant synaptic pruning and significantly lower number of regenerating motoneurons, indicating impaired axon regeneration (114). A similar finding was observed in mice MHC-I knock-out mice, where the sciatic nerve was crushed. The investigators showed that these mice took significantly longer to regain motor function, with some not recovering at all (62). In this regard, it is worth noting that β2m knock-out mice are known to affect normal astrocyte behavior, leading to a decrease in the reactivity of these cells and an increase in astrocytic shrinkage. Moreover, the production of pro-inflammatory cytokines and neurotrophic factors dwindled in this β2m knock-out mice, suggesting a possible neuronal and synaptic dysregulation (115).

In a most interesting study, MHC-I expression and synaptic plasticity was studied in three different mice strains after axotomy: A/J, BALB/CJ, and C57BL/6 mice (116). Of note, these strains differ in the MHC-I alleles they express. The A/J mice express H-2K^{k}, H-2D^{d}, and H-2L^{d} alleles, the BALB/CJ mice express H-2K^{d}, H-2D^{d}, and H-2L^{d} alleles, and the C57BL/6 mice express H-2K^{b} and H-2D^{b} alleles, being null for the H-2L^{d} (117). The study allowed to discern differences in MHC-I expression and synaptic plasticity according to the presence of mice MHC-I alleles. Following axotomy, most changes were noticeable one week after lesion, but with marked differences between strains. A/J mice showed the greater MHC-I expression and astroglial reaction. Both, A/J and BALB/CJ mice exhibited the largest synaptic changes during this timeline, losing a higher number of synapses in the first week after lesion and recovering virtually all of them after three weeks. According to the authors, this could be related to their faster synaptic elimination and recovery. In marked contrast, C57BL/6 mice had the lowest degree of astroglial reaction and,

while they preserved more synapses initially, they failed to recover the rest of them afterwards (116).

When looking at differences between the mice strains, three things are worth mentioning. First, the three mice shared the H-2D^b allele. Second, the three mice differed in the H-2K allele, with A/J having K^k, BALBC/CJ having K^d and C57BL/6 having K^b. Third, C57BL/6 mice, but not A/J and BALB/CJ lacked the H-2L^d allele. On the one hand, the expression of the H-2K^k allele may explain the greater MHC-I expression and astroglial reaction in A/J mice when compared to BALB/CJ mice. On the other hand, the presence of the H-2L^d may explain the better neuroregenerative capacity observed in the A/J and BALB/C mice strains when compared to the C57BL/6 mice strain after axotomy. If correct, these interpretations may suggest that the extent of the neuroregenerative process is influenced by the H-2 haplotype (see below). In that regard, it is important to refer that the mouse H-2L^d allele is known to be more prone to lose β2m and the peptide and become an open conformer than other class I H-2 alleles. This tendency of the H-2L^d allele to dissociate likely results from its peculiar structure. Thus, molecular studies have shown that the α1/α2 domains of the αHC and β2m are oriented in a way that restricts the number of interactions between each part, making the light chain more likely to dissociate. In addition, the H-2L^d allele contains a more hydrophobic peptide-binding cleft than other MHC-I alleles, which in turn could potentiate the rate to which peptides detach from this molecule (118). Accordingly, this allele is more likely to *cis*-associate with receptors that may regulate important events during the neuroregenerative process, as has been demonstrated for other signaling receptors, including receptors for growth factors and immune receptors (119,120).

Further studies have shed light on the different functions that each MHC-I allele may have on neuronal regulation. Mice neurons treated with specific MHC-I antibodies showed a significant increase in neurite outgrowth and neuronal death, especially when the anti-H-2K^b antibody was used (121), which points to this allele as a modulator of neurogenesis, perhaps through interaction with inhibitory Ly49 receptors. Loss of the H-2K^b but not of the H-2D^b allele, resulted in an increased proliferation of neural small progenitor cells (122). These data suggest that H-2K^b molecules may play a more important role than H-2D^b molecules in neurogenesis. Indeed, absence of H-2K^b expression resulted in cell proliferation on mice

hippocampus and was correlated with an increase in the expression of growth factor genes, namely the Fibroblast growth factor receptor 1 gene. Conversely, expression of H-2K^{b} blocked neural small progenitor cell spread by declining Fibroblast growth factor receptor 1 activity (122).

Another study that dwells into this matter examined how humanized mice models with different MHC-I alleles respond to the Theiler's Murine Encephalomyelitis Virus infection and its effects on the CNS (123). While MHC-II and β2m deficient (Aβ^{0}.β2m^{0}) mice did not survive to the Theiler's Murine Encephalomyelitis Virus infection, Aβ^{0}.β2m^{0} mice transfected with human β2m and either with the human MHC-I A11^{+} allele or human MHC-I B27^{+} allele all survived. Strikingly, transgenic human HLA-B27 mice had a notable recovery when compared to their human HLA-A11 counterpart, reflected by their increased capacity of cortical neuron repair and better brain pathological scores. These results are most interesting for one reason. The human HLA-B27 molecule is known for a long time to be associated with the autoimmune disorder Ankylosing Spondylitis and to have a tendency, like the mouse H-2L^{d} allele, to lose the β2m and the peptide and become an open conformer (124,125). Most likely, the human HLA-B27 allele, due to their tendency to become an open conformer and eventually establish *cis*-interactions with themselves (homodimers) or with other receptors (heterodimers), may have a crucial role in neuroregeneration following CNS lesions.

From these studies, it can be concluded that the type of MHC-I molecules (i.e., alleles) expressed by mice and humans could determine the extent of the neuroregenerative process. This influence may also be determined by the differential expression of MHC-I alleles driven by alternative splicing, as recently shown for the various subtypes of mice hippocampal cells (64). The role of classical MHC-I molecules on neuroregeneration can also be extended to non-classical MHC-I molecules, such as Qa-1, which has recently been shown to regulate the function of neural microglia, the main phagocytic cells of the CNS, that are crucial to maintain normal brain homeostasis and synaptic plasticity (57). Thus, the effects of MHC-I molecules on neuronal development and regeneration are enlightening the pathophysiology of cognitive disorders and motor diseases and are opening new

avenues to the development of strategies to slow neurodegenerative diseases or to accelerate neuroregenerative processes.

A brief summary of the findings described in this section can be found in annex IV.

Chapter 5 – HOW MHC-I MOLECULES FINE-TUNE BRAIN HOMEOSTASIS?

Until now, we have provided an overview of the *in vitro* and *in vivo* studies, in animal models and humans, reporting associations between the presence or absence of particular MHC class I molecules and the existence of anomalies in brain development and synaptic plasticity, which usually led to neurodegeneration and the development of cognitive disorders and dementia. However, most studies analyzed neuronal development, synaptic plasticity and synaptic pruning in completely opposite situations. On the one hand, expression of MHC-I molecules was either abolished by a variety of methods, including Short Hairpin RNA, knocking-down and knocking-out MHC-I genes (e.g., H-2$^{-/-}$) or genes involved in their formation (e.g., β2m$^{-/-}$ or TAP$^{-/-}$). On the other hand, expression of MHC-I molecules was markedly increased by gene transfection of specific MHC-I genes, modified MHC-I gene constructs or after treatments that increase MHC-I expression. In either case overt anomalies in brain homeostasis were observed, which strongly point to MHC-I molecules as modulators or fine-tuners of brain function and homeostasis.

Despite the large body of knowledge accumulated, the majority of studies have not provided a clear explanation on how MHC-I molecules influence and regulate brain homeostasis at steady-state levels. In other words, which are the molecular mechanisms behind the associations between the lack or excess of MHC-I molecules and a correct neuronal function and homeostasis? These questions are not easy to answer. However, whatever the answers be they must consider a largely ignored fact: MHC-I molecules, which are constituted by sum of three separate components (i.e., αHC + β2m + peptide), can remain as closed conformers at the plasma membrane or dissociate during specific conditions, generating three components, thus adding more complexity to the understanding of the role of MHC-I molecules in brain homeostasis. This complexity becomes even more entangled if we take into account the fates of the different components.

First, while the αHCs remain embedded in the plasma membrane as open conformers, the β2m and the peptide are released into the extracellular milieu. The fate and possible functions of the released peptides remains unknown. In contrast,

several non-immune roles have been attributed to the plasma membrane open conformers and the soluble β2m.

Second, closed and open conformers at the plasma membrane can also be released into the extracellular milieu embedded in vesicles or as a result of proteolytic cleavage mediated by a membrane metalloprotease, respectively. While soluble closed MHC-I conformers are considered immunoregulatory and capable to suppress immune effector functions, the role of soluble open MHC-I conformers remain uncertain.

Third, both closed and open conformers present at the plasma membrane can self-associate and transiently form homodimers and oligomers, which have been shown to regulate mobility of growth factor receptors and be ligands in *trans* of a variety of NK receptors. Alternatively, the open MHC-I conformers can also *cis*-interact with other receptors and form αHC heterodimers. These interactions have been shown to modulate receptor signaling upon ligand binding, receptor endocytosis and intracellular trafficking.

Therefore, in order to obtain answers to the question of how MHC-I modulate brain homeostasis all the different proteins and alternative forms that can be generated in metabolically active cells from the closed MHC-I conformers must be considered. These include, but are not limited to, the following:

1. Cell surface closed MHC-I conformers and their transient formation of homodimers through *cis*-associations with themselves. These homodimers have been shown to be ligands of activating and inhibitory receptors expressed by NK cells and highly differentiated CD8+ T cells. Some of these receptors are also expressed in neuronal cells (126–137).

2. Cell surface open MHC-I conformers and their transient formation of αHC homodimers through *cis*-associations with themselves. Like above, these homodimers have been shown to be ligands of receptors expressed by different immune cells (126–128,137–144).

3. Cell surface open MHC-I conformers and their transient formation of αHC heterodimers through *cis*-associations with other receptors. These

heterodimers are constituted by αHCs and another receptor. Among these receptors, the following have been identified: the insulin receptor, the epidermal growth factor receptor, the IL-2/IL-15 receptors, the luteinizing hormone receptor, and neurotransmitter receptors, such as the receptor for γ-endorphin, the β-adrenergic receptor and the acetylcholine receptor. All these *cis*-associations have been shown to modulate cAMP levels and other signaling pathways upon ligand binding (15,119,145–156).

4. Soluble closed MHC-I conformers released in vesicles. The closed MHC-I conformers of these vesicles may function as signaling molecules on the post-synaptic axons upon interactions with their ligands (157–164).

5. Soluble open conformers released after the action of metalloproteases. The free open MHC-I conformers may also function as signaling molecules on the post-synaptic axons upon interactions with their ligands (165,166).

6. Soluble β2m released after dissociation of the closed conformers. The soluble β2m may play a variety of functions depending on the structure it associates with (106,167,168).

The accumulated body of evidence from experimental and clinical studies strongly indicates that any of these alternative forms, either alone or as compounds with other receptors or even with themselves, should underlie these associations. In this respect, it is important to emphasize that in most of the studies using animal models MHC-I molecules are found: 1) in the plasma membrane (although at low or null levels perhaps due to molecular associations with other receptors and proteins that may mask their detection); 2) in intracellular compartments and granules; 3) in intracellular vesicles present in pre- and post-synaptic axons; and 4) secreted into the synaptic cleft by presynaptic neurons.

Overall, these data strongly suggest that the role or roles of MHC-I molecules expressed in neurons are beyond immune recognition and more related with the physiology of the cells of the brain parenchyma. These biological aspects are critical to try to delineate the possible mechanisms whereby MHC-I molecules modulate brain homeostasis. Several issues can be proposed and discussed and, certainly, all are interconnected.

5.1. Neuronal integrity and survival may rely on the equilibrium between closed and open MHC-I molecules and their secretion

Closed MHC-I conformers may have a predominant role in fine-tuning proper neuron development. Retinal mice tissue cultured with soluble H-2D^b molecules loaded with the H13a peptide had a decrease in neurite outgrow, suggesting that MHC-I molecules may have neuroinhibitory properties (165). When self MHC-I peptides were present, the inhibitory effect on neurite growth was stronger than when their non-self-counterpart was not present, which implies that neurons may have a preferable response to self-MHC class I allele products. Noteworthy, this reduction in neurite outgrowth was dependent on the proper conformation of MHC-I molecules. By subjecting fully closed MHC-I conformers to both heat treatment to 65ºC and preabsorption with conformation dependent antibodies, it was revealed that there was a disruption in the MHC-I conformation and in the initial inhibitory effects. In another curious note, exogenous MHC-I peptides did not affect MHC-I knockout mice, pointing to the possibility that neuron sensibility to these molecules is dependent on the correct endogenous MHC-I expression. Therefore, it can be suggested that correct MHC-I expression in their closed conformer state is needed for proper neurodevelopment by inhibiting excessive growth. This line of reasoning can be found in another study that showed that embryonic mouse retina explants cultured near thalamic explants derived from mice overexpressing H-2D^d molecules had their neurite outgrowth diminished, being this effect reverted after the addition of a conformation dependent anti-H-2D^d monoclonal antibody (166). A similar result was seen when embryonic mouse retina explants were cultured near monkey COS cells modified to express either membrane bound H-2D^d molecules or in their soluble form. Surprisingly, addition of the protein kinase A inhibitor Rp-cAMP reverted blockage in neurite outgrowth exerted by soluble H-2D^d molecules significantly (166). Therefore, besides confirming the potential role of cell membrane bound MHC-I closed conformers in the regulation of neurite outgrowth, soluble MHC-I molecules (sMHC-I) can also exert neuroinhibitory properties, possibly acting through a modulation in the cAMP levels of neurons. Since it was already demonstrated that mice neurons from the dorsal root ganglia cultured with H-2D^b monomers had a drop in neurite outgrowth (169), perhaps, the presence of closed

MHC-I conformers in vesicles secreted in the synaptic cleft may suggest that these forms participate in the transmission of the electrochemical signal between neurons, thus functioning, either as fine-tuners of the known neurotransmitters or as neurotransmitters themselves.

One noteworthy study revealed that stimulated human purified T and B cells showed an increase in the secretion of soluble HLA-I molecules, suggesting that the secretion of sMHC-I is a general property of metabolically active cells that may have physiological and clinical relevance. When these results are taken into account, it is not unlikely to think that neurons may also be capable of secreting sMHC-I molecules in certain conditions. Although we do not currently know if neurons are capable of secreting sMHC-I molecules, nor in which conditions this may occur, it is possible that inflammation and, at some extent, neuronal activity could promote the neuronal secretion of sMHC-I molecules in order to counteract the inflammation, neuron loss and possible neurodegeneration.

The soluble β2m may play a role in the regulating the balance between closed and open MHC-I conformers. β2m may bind to open MHC-I conformers inducing the formation of closed conformers again. Incubation of different human cell lines with soluble human β2m resulted in an increased staining of the W6/32 antibody (specific for MHC-I closed conformers) and it decreased the staining of the L31 antibody (specific for MHC-I free heavy chains), revealing that the presence of β2m does indeed promote a shift towards the formation of MHC-I molecules in their classic form (138). However, this effect was not seen in cells that were not able to express either β2m or surface HLA-I molecules. Therefore, it is possible that neuroinflammation could also lead to the formation of open MHC-I conformers and consequent secretion of β2m, which could explain the already described increased β2m contents in AD patients and in the elderly (108–110). What is the fate of this free soluble β2m? Two possibilities can be proposed: either it promotes the formation of MHC-I closed conformers, preventing neuron impairment; or it induces the formation of Aβ plaques, which may explain its amyloidogenic property (106,167,168). It is also plausible that these two possibilities could co-exist. In other words, throughout life, as the neurons of the brain become more susceptible to inflammation and to morphological changes, which can be reflected by an excessive

neurite outgrowth, they release either β2m to promote closed MHC-I conformer formation or vesicles containing fully conformed sMHC-I molecules in order to assure proper neuronal adaptation. However, during aging, or even chronic neurodegeneration, this may not be sufficient. In a desperate move to try to contain the consequent neuroinflammation, neurons may release excessive amounts of β2m that, ultimately, become prone to forming amyloidogenic plaques.

5.2. MHC-I conformers may constitute an alternative approach for neurosignalling

Another way in which MHC-I molecules may regulate neuronal function is through the formation of MHC-I open conformers and direct *cis*-association with its ligands. To our knowledge the following MHC-I ligands have been found to be expressed in the nervous system: PirB, Ly49, LILRBs and KIRs (170).

Ly49 NK receptors were found to be expressed in various mice brain regions, such as the cerebral cortex, hippocampus and cerebellum. It was observed that mice primary embryonic cortical neurons expressed both MHC-I and Ly49 proteins in their somas and axons, with both being associated with synaptic markers. Interestingly, while staining of MHC-I molecules revealed that they were mainly present in neurites, Ly49 was prominently present in the cell bodies and axons. Furthermore, when these neurons were treated with specific anti-H-2K^{b} and H-2D^{b} antibodies, there was an increase in neurite outgrowth and a reduction in neuron survival. In contrast, the use of the Ly49 antibody had the opposite effect: it improved neuron survival and decreased the neurite outgrowth (121). Therefore, it could be speculated that, in the synaptic cleft, in order to promote neuronal survival and proper functioning, pre-synaptic MHC-I molecules should interact with post-synaptic Ly49 proteins. Considering the inhibitory nature of Ly49 proteins on cell function, the lack of interaction between Ly49 and MHC-I molecules could hinder the quantity and quality of signals that the neuron receives, which may impair its function and lead to neuronal loss trough “death signals”. Although this interaction could occur across two different cell membranes, *cis*-interactions between Ly49 and MHC-I having neuronal influence should not be ruled out as they also exist. In an immunological context, some studies have confirmed that *cis* and *trans* interactions between Ly49 proteins and H-2D^{d} molecules do occur, influencing cell susceptibility to cytotoxic

lysis by NK cells and CTLs. From these findings, a model that explains this relationship has been proposed: the Ly49 receptor adopts a back-fold conformation to interact in *cis* and an extended form to associate in *trans* (130,133). It is, thus, possible that the presence of MHC-I molecules (closed or open) at the pre-synaptic active neurons, either at the plasma membrane or in vesicular compartments, may contribute to translating electrical activity into a chemical inhibitory signal, and therefore into changes in synaptic strength and neuronal connectivity.

Another MHC-I ligand that may have a role in neuron regulation is the inhibitory PirB receptor, which corresponds to murine ortholog of the human LILRB1 and LILRB2 receptors. In healthy mice brains, the PirB protein was found to be expressed in various regions linked to synaptic communication, namely in the hippocampus and cerebellum, and in the soma from neurons of these same areas (68,171). Mice with mutations on this protein had an increase in visual plasticity and dominance during development (171), suggesting that that *trans*-interactions between MHC-I molecules and PirB receptors could also be involved in neuronal plasticity. Indeed, in another study that corroborates these findings, it was discovered that knockout of the PirB protein had an impact in the morphology of mice neurons from the visual cortex with a significant increase in dendritic spine density and decrease in their motility. Moreover, these changes had a functional impact with these neurons having higher excitatory synaptic connectivity and strength (172). Another noteworthy study in relation to this possible function observed that mice lacking the PirB protein lost their asymmetrical hippocampal circuitry, being this effect similar to what was seen in β2m knockout mice, suggesting that MHC-I molecules may regulate proper neuron development through the inhibitory signals transmitted by PirB (173).

One curious aspect that needs to be addressed is the finding that PirB proteins were gradually more expressed with aging, being particularly present in aged mice with cognitive impairment (68). Thus, in neurodegenerative diseases, such as AD, PirB can be also a key player in AD pathogenesis. Oligomerized $A\beta_{42}$ plaques were found to bind to this receptor and PirB expression removal attenuated the deteriorating effects that these plaques had on the hippocampus and memory in AD mice models (174). Perhaps, in this context, the presence of the PirB receptor may have a detrimental effect on brain function since its role is mainly related to inhibition of

cell function. As one study found, among the myelin sheet that involves axons of neurons and is responsible for neurotransmission, PirB was shown to bind to inhibitory myelin receptors and weaken axonal regeneration. Interestingly, genetic removal of PirB or administration of specific PirB anti-bodies reverted this inhibition (175). This finding may have clinical significance since impairing the myelin sheet formation hinders to ability of the neuron to transmit and receive signals, possibility leading to neuronal death. In fact, this can explain why, following stroke, mice lacking either PirB or MHC-I molecules had not only a smaller infarct brain area, but also a quicker recovery and less sequels than their WT counterpart (176). Indeed, in this stroke model, PirB knockout mice had a higher cell survival and lower astrocyte activation, possibly indicating that the degree of neuroinflammation was less severe (176). Therefore, these results point to the notion that low and regulated levels of brain PirB protein could contribute to neuroprotection.

One interesting question arises from this observation: why neurons would express such receptor that is harmful to neuroregeneration? Perhaps, the initial expression of PirB is not to cause harm, but to assure proper neuron refinement. However, chronic inflammation may cause neuronal stress that leads to excessive PirB expression, further perpetuating this neurodegenerative state. It is also possible that the detrimental effects of PirB could be related to individuals having specific MHC-I alleles more susceptible to cognitive impairment, a reasoning that can be extrapolated to LILR receptors, the human counterpart of Pir receptors.

In fact, it has been shown that some LILR receptors have a preferential binding to HLA-I molecules, particularly in their non-classical form. Soluble LILRB1-Fc fusion proteins showed a preference to associate with HLA-A2, HLA-A3, HLA-B7, and HLA-B27 molecules, as these alleles contained cysteine residues important for HLA-I dimerization (such as Cys325 and Cys339) (136) . Thus, the LILRB1 receptor may have a preferential binding to HLA-I dimers. In relation to the LILRB2 receptor, it has been found that it has a higher affinity for HLA-B27 homodimers and free heavy-chains than for HLA-B27 heterotrimers (i.e: closed conformers), meaning that is possible that HLA-B27 molecules may bind to two LILRB2 receptor molecules (134). Hence, these ligands may prefer binding with open HLA-I conformers in order to further promote the inhibitory signaling needed for regulating excitatory synapse plasticity and development. Thus, aging and neuroinflammation may induce the

formation of MHC-I open conformers with the goal of promoting its association to inhibitory LILR receptors in order to control the degree of neuronal stress and, perhaps, neurodegeneration.

Although the previous findings suggest that MHC-I molecules bind preferentially in their open conformer to inhibitory ligands, they could also bind to excitatory molecules. Indeed, pull-down experiments using KIR3DS1-His incubation only resulted in the detection of cells expressing MHC-I open conformers, suggesting that KIR3DS1, a excitatory receptor, molecules may have a higher affinity for these types of MHC-I molecules (143).

Unexpectedly, hormone receptors may also be capable of interacting in *cis* with MHC-I molecules with the goal of regulating neural signal transduction, as it has been shown that MHC-I molecules were capable of forming complexes with the EGF and insulin receptors (146–148,154,177). In a peculiar relation with the insulin receptor, in the hippocampus of mice lacking MHC-I molecules, it was demonstrated that there was an increased activation of the insulin receptor signaling and phosphorylation of its mediators (177). Moreover, the increased hippocampal synaptic density in MHC-I knockout mice was correlated and restricted to certain brain regions where the insulin receptors were expressed, for example, in the CA3 hippocampal region. Interestingly, this increase in synaptic content could be reverted by using rapamycin, an inhibitor of the insulin signaling pathway, pointing to a possible important connection between MHC-I molecules and insulin receptors on the regulation of synaptic genesis. In hippocampal neurons derived from MHC-I knockout mice brain, although the insulin receptor levels were normal, the C19 monoclonal antibody, which targets the β chain of the insulin receptor, failed to detect this receptor. Surprisingly, C19 staining was rescued when these neurons were incubated with normal WT MHC-I expressing neurons, but not with soluble β2m. In this respect, it is important to note that, in mice hippocampal neurons expressing MHC-I, insulin receptors were primarily expressed in the axons, whereas MHC-I was found in dendrites, thus suggesting that MHC-I molecules might influence insulin receptor signaling in neighboring cells through nearby interactions and changes in the conformation of insulin receptors (177). The likelihood that this interaction could depend on the formation of MHC-I open conformers remains to be ascertained.

Chapter 6 – FUTURE PROSPECTS

This thesis work tried to draw attention into the role of MHC-I molecules in the normal brain function and their implication on the development of cognitive impairment, either by aging or by neurodegeneration. However, one crucial aspect that still needs to be answered is how all this information can have a clinical impact.

In relation to this issue, several lines of study can be proposed, namely in humans. Firstly, it would be interesting to know how the neuronal expression of HLA-I molecules changes in the normal healthy brain through aging and how that translates into the formation of closed and open HLA-I conformers. Secondly, if neurons are capable of expressing membrane bound HLA-I molecules, it may be relevant to discover what triggers their secretion in the form of soluble HLA-I molecules, or even if this is possible, and what is its role on brain function. The expression of HLA-I ligands (e.g., LILR, KIR) could also be investigated. On a curious note, it is possible that these ligands may have specific interactions with closed or open HLA-I conformers, having neurological implications. Finally, it would be interesting to further develop on which HLA-I alleles are more prevalent in patients either with AD, PD or cognitive impairment. In this context, it would be worth it to study a well-represented cohort of patients with either one of these conditions and analyze the amount of β2m and the presence of membrane bound or soluble HLA-I conformers, both closed and open, on the CSF and the brain parenchyma, using non-invasive techniques, on these patients. In these individuals, the proportion of closed to open HLA-I conformers could also be explored in an attempt to correlate it with the buildup of neuroinflammation, while also looking for potential connections, such as misfolding of Aβ plaques and loss of dopaminergic neurons.

The truth is that, at the time of writing, none of these possibilities have been fully explored. Thus, in the future, it may be relevant to further analyze this understudied area of research, which may lead to new exciting clinical possibilities and give answers to the control and treatment of cognitive disorders.

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Annexes

Annex I - Brief summary of the findings described in section 3

Authors	Year	Cells/Tissues/Organism	Results	References
Lampson and Fisher	1984	Cell lines derived from Neuroblastoma	All neuroblastoma cell lines showed increased β2m and W6/32 activity with IFN-γ treatment compared to untreated controls. There was no increase in MHC-II molecules. There was increased expression of both component chains of MHC-A, B, C in IFN-γ treated cells.	(38)
Lampson and George	1986	Cell lines derived from Neuroblastoma (IMR-5, NMB, CHP-126)	IFN-γ treatment increased MHC-I RNA levels in all three neuroblastoma cell lines. Untreated cell lines showed weak basal levels of β2m RNA. IFN-γ treatment increased β2m RNA levels in IMR-5 and NMB cell lines. Both HLA-A and HLA-B polypeptides were expressed after IFN-γ treatment in IMR-5, NMB, CHP-126, CHP-100, and CHP-134 cell lines.	(39)
Mauerhoff *et al*	1988	Human fetal brain	In a basal cell state, HLA-I molecules were present on astrocytes, but not on oligodendrocytes or neurons. IFN-α, IFN-γ, and TNF-α increased HLA-I expression in astrocytes and fibroblasts. Oligodendrocytes acquired HLA-I expression with IFN-γ or TNF-α treatment. Neurons remained HLA-I negative.	(37)
Neumann *et al*	1995	Neurons from hippocampal cultures of Lewis mice	β2m mRNA was detected in 1 out of 10 active and 1 of 11 silent neurons. MHC-I heavy chain mRNA was detected in 3 of 10 active and 8 of 11 silent neurons. Only 1 neuron in each group expressed both simultaneously. IFN-γ increased MHC-I and β2m expression in silent neurons. 6 of 12 silent neurons expressed β2m, and 11 of 12 expressed MHC-I heavy chain mRNA. All neurons treated with Tetrodotoxin and IFN-γ expressed both β2m and MHC-I mRNA. Immunofluorescence labeling and confocal microscopy showed that some IFN-γ treated neurons expressed MHC-I molecules on their surface. In the presence of Tetrodotoxin, almost all neurons expressed surface MHC-I molecules.	(40)
Neumann *et al*	1997	Neurons from hippocampus tissue of fetal Lewis mice	In untreated neurons, 12/22 had MHC-I heavy chain mRNA, 2/22 had β2m, and 0/22 had TAP1/TAP2 mRNA. Astrocytes consistently had MHC-I heavy chain and β2m mRNA; TAP1 and TAP2 transcripts were present in some astrocytes. IFN-γ did not alter bioelectric activity. 13/20 neurons had MHC-I mRNA, 7/20 had β2m, and 5/20 had TAP1/TAP2	(42)

			mRNA. Tetrodotoxin treatment with IFN-γ induced MHC-I in almost all neurons. Glutamate reduced IFN-γ and Tetrodotoxin induced MHC-I gene transcription to baseline levels. No surface MHC-I molecules on untreated neurons. IFN-γ treatment induced MHC-I surface expression in some of neurons. IFN-γ and Tetrodotoxin treatment induced expression in 91% of neurons, reduced to 32% with additional glutamate. TNF-α did not alter electrophysiological parameters. It induced MHC-I heavy chain mRNA in 20/20 neurons but did not enhance β2m or TAP1/TAP2 transcription. No surface MHC-I was detected.	
Corriveau *et al*	1998	Cats Long-Evans mice	MHC-I mRNA expression levels changed during specific developmental stages in the cat visual system, coinciding with synaptic plasticity. There was presence of β2m, MHC-I and CD3ζ mRNA in the developing cat lateral geniculated nucleus. MHC-I mRNA and protein expression was detected in the somatosensory cortex and hippocampus of mice at the 22-week postnatal development period. Kainic acid-induced seizures increased MHC-I mRNA expression in the dentate gyrus of the hippocampus and superficial layers of mice neocortex.	(44)
Lindå *et al*	1999	Sprague–Dawley mice	Mice brainstem neurons, including motoneurons and nigral dopaminergic neurons, expressed both MHC-I and β2m mRNA, being this expression varied across different nuclei. IFN-γ receptor mRNA expression was low but significantly elevated in mice brainstem neurons. Dopaminergic neurons in the mice substantia nigra compacta exhibited particularly high expression of β2m.	(53)
Huh *et al*	2000	C57BL/6 wild-type mice β2m knockout and deficient mice TAP1 knockout mice CD3ζ knockout mice RAG1 knockout mice	MHC-I and CD3ζ transcripts were present in developing murine CNS, with distinct expression patterns among neuron subsets. Genetic analysis in mice lacking MHC-I or CD3ζ revealed altered retinal projections and synaptic plasticity. Mice lacking either β2m, TAP1, MHC-I or CD3ζ showed a shift towards synaptic potentiation in response to neural activity.	(45)
Goddard *et al*	2007	C57BL/6 wild-type mice hippocampal tissue β2m/TAP1 knockout mice hippocampal tissue	Punctate MHC-I protein immunostaining was observed in soma and dendrites in hippocampal neurons. Abnormal basal synaptic transmission and structural changes in synapses in β2m and TAP1 knockout neurons and in neurons treated with Tetrodotoxin, showing increased mini-excitatory postsynaptic currents and synapsin immunoreactive boutons.	(56)

Datwani *et al*	2009	C57BL/6 wild-type mice β2m/TAP1 knockout mice H-2K^b and H-2D^b knockout mice	Enhanced Ocular Dominance Plasticity and abnormal retinogeniculate patterning in H-2K^b and H-2D^b knockout mice. Abnormal Segregation of Eye-Specific Inputs in the dorsal lateral geniculate nucleus of H-2K^b and H-2D^b knockout and β2m and TAP1 knockout mice. MHC-I was localized near synapses in mice lateral geniculate nucleus, closely associated with postsynaptic density protein 95 and synapsin puncta.	(58)
Thams *et al*	2009	C57BL/6 wild-type mice β2m/TAP1 knockout and deficient mice H-2K^b and H-2D^b knockout mice	Upregulation of MHC-I and β2m mRNAs in the wild-type mice spinal cord after axotomy. H-2D^b immunoreactivity was observed in untreated and IFN-γ treated in the axons of wild type mice MN1 cells, a motoneuron-like cell line. Presence of H-2D^b immunoreactivity at neuromuscular junctions in intact and reinnervated muscles of wild type mice was confirmed by confocal microscopy. H-2K^b and H-2D^b knockout mice showed altered synaptic band structure and increased density of acetylcholine receptor clusters in hindlimb muscles and diaphragm. Delayed recovery of motor function after sciatic nerve crush in H-2K^b and H-2D^b knockout mice compared to wild type. Decreased covering of terminal Schwann cells at the neuromuscular junctions in H-2K^b and H-2D^b knockout mice.	(62)
Needleman *et al*	2010	Visual Cortex of Long–Evans mice aged P7, P23, and adult	Immunohistochemistry of the rat visual cortex revealed that MHC-I protein expression increased significantly from the 7 to 23-week postnatal stage and adult stages. Densely punctate MHC-I staining was observed on mice proximal dendrites at all ages. Presence of MHC-I immunostained puncta in neuropil suggested dendritic and/or axon terminal staining. Throughout development, in the mice layer V visual cortex, most MHC-I proteins were localized to synaptic structures and dendrites. MHC-I proteins were present pre- and postsynaptically at all ages examined. Association of MHC-I proteins with synaptic vesicles, synaptic cleft, and postsynaptic densities was also observed.	(54)
Wu *et al*	2011	C57BL/6 wild-type mice NSE-D^b mice β2m deficient mice	Contralateral retinogeniculate projections and total dorsal lateral geniculate nucleus area were significantly smaller in NSE-D^b mice compared to wildtype. β2m deficient mice had larger ipsilateral dorsal lateral geniculate nucleus area compared to wild-type mice. Synaptophysin immunostaining was significantly lower in various hippocampal regions of NSE-D^b mice compared to wildtype. Decreased width and number of neurons across the pyramidal cell layer in CA1 of NSE-D^b mice.	(51)

			NSE-D^b mice showed inhibited compensatory sprouting response and delayed recovery in hippocampal-dependent spatial task following unilateral perforant path lesioning compared to wildtype mice.	
Glynn *et al*	2011	Neurons from the occipital cortex of newborn C57BL/6 wild-type mice Neurons from the occipital cortex of newborn β2m knockout mice	MHC-I proteins were present in synaptosomes of the adult mice CNS, colocalized with postsynaptic density protein 95 in cultured hippocampal neurons, and were found in both axon terminals and postsynaptic densities of synapses in the mice visual cortex at multiple ages. MHC-I proteins were present on the surface of all examined neurons in clusters before, during, and after the peak of synaptogenesis, including on axons and dendritic growth cones. Knockout of surface MHC-I proteins increased synapse density, while overexpression decreased it. β2m knockout mice showed substantially higher synapse density in the visual cortex at different ages compared to wildtype mice. Decreasing homologous surface MHC-I clusters increased glutamatergic synapse density. In addition, blocking neural activity with Tetrodotoxin decreased surface MHC-I levels, leading to an increase in glutamatergic synapse density, being this effect mitigated by MHC-I overexpression.	(55)
Liu *et al*	2013	C57BL/6 J mice	Both H-$2K^b$ and H-$2D^b$ mRNA expression were present in various brain regions throughout mice development. H-$2D^b$ protein overlapped with Neuronal Nuclear antigen-positive neurons in mice hippocampus, cerebral cortex, and olfactory bulb.	(50)
Chacon and Boulanger	2013	C57BL/6 mice	MHC-I expression was detected in coronal and parasagittal sections of mouse embryos at mid-gestation, being localized in both cytosolic and membrane compartments. MHC-I co-expressed with various markers of neural progenitors in mice prenatal brain.	(52)
Zhang *et al*	2013	Human brain tissues	HLA-I heavy chain protein expression was detected in the developing hippocampus, dentate gyrus, subiculum and entorhinal cortex, being absent in adults except in blood vessels. β2m expression was present in the developing hippocampus, dentate gyrus, and subiculum, being absent in the entorhinal cortex except in blood vessels. HLA-I heavy chains co-expressed with Neuronal Nuclear antigen-positive and with glutamatergic neurons in the developing hippocampus, dentate gyrus, and subiculum. Not in astrocytes or microglia. There was no co-localization with Gamma-Aminobutyric Acid.	(59)
Lv *et al*	2014	C57BL/6J mice Human brain tissues	H-$2K^b$/D^b proteins were expressed by the developing mice Purkinje cells and cerebellum.	(60)

			Expression of HLA-B, HLA-C and β2m was increased in the developing human cerebellar cortex, being absent in adults.	
Zhang *et al*	2015	Human brain tissues	HLA-I protein expression was detected in the developing human cerebral and cerebellar cortex, brainstem and spinal cord. HLA-I mainly localized postsynaptically, overlapping with the postsynaptic density protein 95 in the human lateral geniculate neurons, Purkinje cells, and cochlear nuclei.	(61)
Tetruashvily *et al*	2016	C57B1/6 wild-type mice β2m/TAP1 knockout mice H-2K^{b} and H-2D^{b} knockout mice NSE-D^{b} mice	β2m and TAP1 deficient mice diaphragm showed increased multiple-innervated muscle fibers and quantal size at neuromuscular junctions compared to wild-type mice. Wild type mice developing neuromuscular junctions injected with anti-MHC-I antibodies had increased multiple innervations. While the NSE-D^{b} mice diaphragm had a decrease in multiple-innervation, mice lacking H-2K^{b} and H-2D^{b} had an increase in multiple innervations. In wild-type mice, MHC-I proteins were present in the developing neuromuscular junctions, primarily co-localizing with postsynaptic nicotinic acetylcholine receptors. MHC-I protein levels increased in the aging mice neuromuscular junction. H-2K^{b} and H-2D^{b} knockout mice aged mice exhibited reduced muscle fiber denervation compared to wild-type mice.	(63)
Tetruashvily *et al*	2016	C57BL/6J mice	MHC-I mRNA expression was detected in mature mice motor neurons and at the adult neuromuscular junction. MHC-I mRNA expression increased with aging, varying between muscles (diaphragm, extensor digitorum longus, and soleus). MHC-I proteins were detected at the developing mice neuromuscular junction at the 7-, 15- and 30-week postnatal development period, co-localizing with synaptophysin and nicotinic acetylcholine receptors. MHC-I and MHC-I-like genes were expressed in the developing mouse hippocampus. Several MHC-I spliced variants were found in the hippocampus, including the Q-10 lacking the α2 domain and MR1 with retained introns. No splice variants were detected at the neuromuscular junction.	(64)
Marin *et al*	2022	Qa-1^{R72A} [26] mice Qa-1^{KO} [25] mice	Qa-1 mRNA expression was detected primarily in the layer 6 of corticothalamic neurons, being enriched in synaptosomes and strongly colocalized with excitatory pyramidal neurons. There was no expression with markers for inhibitory neurons or glial cells. In the visual cortex, Qa-1 mRNA was not detected before eye opening but increased post-eye opening, peaking during the visual critical period.	(57)

			Qa-1 knockout mice exhibited greater ocular dominance plasticity after monocular deprivation. The effect was similar in mice with a mutation in the Qa-1 receptor interaction domain. CD94/NKG2 Qa-1 receptors, specifically NKG2C, were detected in cortical microglia but not in neurons. Microglia engulfed fewer Qa-1 knockout synaptosomes compared to wild-type mice. Microglia in Qa-1 knockout mice did not increase process ramification in response to monocular deprivation, unlike in wild-type mice.	
Lafourcade *et al*	2022	C57/BL6 mice	Monosynaptic rabies tracing in C57/BL6 mice showed that retrosplenial cortex receives long-range inputs from ipsilateral ipsilateral primary visual cortex, secondary motor cortex, and lateral dorsal thalamus, with synaptic connections favoring specific dendritic zones of L5b pyramidal cells. Ipsilateral primary visual cortex inputs synapse at basal and proximal oblique dendrites, secondary motor cortex at the proximal trunk and oblique dendrites, and lateral dorsal thalamus at the distal apical dendritic tufts. Basal branches show supralinear integration, distal tuft dendrites display moderate supralinear integration, and proximal oblique branches exhibit mostly linear integration due to high AMPA:NMDA receptor ratios. Basal dendrites exhibit strong NMDA receptor-mediated supralinear summation, contributing to large local calcium signals before action potential initiation. Subcellular compartment-specific differences in AMPA:NMDA ratios underpin distinct integration modes across dendritic domains. Experimental reduction of AMPA:NMDA in oblique dendrites induces supralinear integration.	(41)

Annex II - Brief summary of the findings described in section 4.1

Authors	Year	Cells/Tissues/Organism	Results	References
Zhang *et al*	2010	Primary human aortic endothelial cells	Stimulation of human endothelial cells with either with W6/32 or F(ab)2 fragments of W6/32 promoted the formation of MHC-I complexes with integrin β4, but not with other integrins such as β1,3 or 5. Knockdown of integrin β4 using Small Interfering RNA inhibited HLA-I-mediated phosphorylation of signaling proteins (Src, Akt, ERK). Both MHC-I traditional molecules and integrin β4 were found colocalized in a punctuate patter on the basal membrane of human endothelial cells. Recombinant mutant HLA-A2 molecules with either the cytoplasmic or extracellular domain deleted showed that only the intact cytoplasmic domain allowed association with integrin β4. Endothelial cells transfected with Small Interfering RNA against the MHC-I heavy chains and against β2m inhibited laminin-5-induced phosphorylation of ERK protein. Integrin β4 knockdown inhibited HLA-I-mediated endothelial cell proliferation. Transfected endothelial cells with Small Interfering RNA against β2m, HLA-I heavy chains and integrin β4 and then treated with mitomycin C had decreased migration capacity in comparison to cell with Small Interfering RNA controls. Plated on laminin 5 and stimulated with bFGF, endothelial cells transfected with Small Interfering RNA against integrin β4 and HLA-I heavy chains had their migration across an artificial wound blocked.	(73)
Yang *et al*	2013	Human Brain Microvascular Endothelial Cells (HBMECs) BV2 microglia cell line	$A\beta_{1-42}$ injection into rat hippocampus induced brain microvascular endothelial cells to express higher levels of MHC-I compared to $A\beta_{42-1}$ injection. $A\beta_{1-42}$ directly exposed to HBMECs did not affect MHC-I expression. However, supernatant from $A\beta_{1-42}$-treated BV2 microglia increased MHC-I expression on HBMECs in a time-dependent manner. The migratory rate of T cells was elevated with culture supernatant from Aβ-treated BV2 in the lower chamber of Transwell inserts. Anti-MHC-I antibody blocked T cell migration through the blood brain barrier model, and T cell adhesion to HBMECs increased with Aβ-treated BV2 supernatant, which was abolished by anti- MHC-I antibodies. MHC-I expression was decreased using MHC-I Small Interfering RNA in HBMECs, reducing T cell transmigration through the HBMEC monolayer and decreasing T cell adhesion when supernatant from $A\beta_{1-42}$-treated BV2 cells was added.	(71)

			Incubation of $A\beta_{1-42}$-treated BV2 supernatant with anti-TNF-α antibodies before adding to HBMECs significantly reduced MHC-I expression on HBMECs at both transcript and protein levels. Anti-TNF-α antibodies blocked both T cell adhesion to HBMECs and transendothelial migration induced by $A\beta_{1-42}$-treated BV2 microglia supernatant.	
Cebrián *et al*	2014	Healthy human brain tissues Brain tissues of patients with Parkison Disease (PD) Wild-type C57BL/6, OT-1, OT-2 and B6.129P2-$B2m^{tm1Unc}$/J mice	In catecholamine neurons, MHC-I presence was confirmed by using immunofluorescence, immunoperoxidase, and immunoelectron microscopy on control human porstmortem entorhinal cortex and striatal sections. In patients with PD, MHC-I had a similar distribution, but generally reduced expression. Using immunofluorescence in the hippocampus and caudate nucleus from human controls, MHC-I was detected only in blood vessels, not in neurons or glia. No MHC-II was detected in neurons. Using immunofluorescence in the Substantia Nigra (SN) and Locus Coeruleus (LC) from human controls, MHC-I was present in many tyrosine hydroxylase (TH)+ neuromelanin (NM)-containing neurons and blood vessels. MHC-I expression was reduced in PD patients. No MHC-I in ventral tegmental area (VTA) dopamine neurons. Immunoperoxidase labeling confirmed MHC-I presence in NM+ SN and LC neurons of controls and PD individuals, but not in NM- neurons or glia. Mass spectrometry detected MHC-I and β2m proteins in isolated NM organelles and purified NM from control human SN. No MHC-II peptides were found. mRNA analysis showed robust expression of β2m, HLA-A, and HLA-C genes in NM+ SN neurons from control human samples. CD8+ CTLs were observed near HLA-expressing NM+ neurons in SN and LC in PD patients, often at higher levels than in healthy patients. MHC-I expression induced by IFN-γ in TH+ neurons from VTA, SN, and LC. MHC-I null neurons did not express MHC-I upon IFN-γ treatment. IFN- γ secreted by activated microglia (induced by NM, α-syn, LPS) led to MHC-I display in ventral midbrain (VM) neurons. Neutralizing IFN-γ reduced MHC-I expression by 30-50%. High cytosolic DA, induced by L-DOPA, led to NM formation and MHC-I expression in mice SN neurons. Combination of CTLs, IFN-γ, and SIINFEKL induced significant death in mice TH+ neurons. Inhibition experiments indicated involvement of Fas/Fas ligand and perforin/granzyme pathways. Microglial activation compounds further enhanced CTL-mediated neuronal death.	(79)
Gate *et al*	2020	Brain tissue, plasma, peripheral blood mononucleated cells	Detected increased CD8+ effector cells and decreased memory cells in PBMCs from MCI or AD patients.	(72)

		(PBMCs) and cerebrospinal fluid (CSF) from healthy individuals, individuals with mild cognitive impairment (MCI), individuals with AD and individuals with PD APP/PS1 mice expressing a chimaeric mouse/human mutant amyloid precursor protein and a mutant human presenilin 1 protein	Found negative correlation between CD8+ TEMRA cells and cognition in MCI and AD. CD8+ TEMRA cells from patients with MCI or AD expressed higher levels of MHC genes, specifically human leukocyte antigen C (HLA-C) and β2m,compared to control cells. Detected CD8+ T cells in AD brains, particularly around cerebral amyloid angiopathy and Aβ plaques. Found clonally expanded CD8+ T cells in CSF from patients with AD, with increased cytotoxic effector gene expression.	
Zalocusky *et al*	2021	Wild type mice β2m knockout mice ApoE knockout mice ApoE3-knock in mice ApoE4- knock in mice ApoE3- knock in/Syn-Cre mice ApoE4- knock in/Syn-Cre mice Religious Order Study, the Memory and Aging Project (ROSMAP) cohort	In aged ApoE knock in mice, neuron-specific knockout of ApoE protected against neuronal and synaptic loss and hippocampal volume reduction. This intervention also reduced MHC-I expression. Reducing MHC-I expression in mice neurons with short hairpin RNA-β2m lentivirus transfection alleviated tau pathology in vitro and in vivo. ApoE expression levels were higher in β2m knockout and wild-type neurons compared to ApoE knockout neurons. P-tau mislocalization, both to the neuronal soma and dendrites, was significantly reduced in β2M- knockout and ApoE knockout neurons compared to wild-type neurons. Injection of AAV2 expressing human pathological tau (tau-P301S) into the hippocampi of wild-type and β2M- knockout mice showed that functional reduction of MHC-I ameliorated induced tau pathology.	(75)
Wang *et al*	2021	Human neuroblastoma SH-SY5Y cells C57BL/6 wild-type mice	MHC-I expression increased in SH-SY5Y cells post Methyl-4-Pheylpyridinium or IFN-γ treatment, dose- and time-dependently. In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine -treated mice, dopaminergic MHC-I positive neuronal loss began 1 day post-injection, with significant loss and morphological changes by 7 days. PD model mice showed MHC-I expression and slight T cell infiltration 1 day post 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine injection, increasing significantly by 7 days. MHC-I gene silencing via stereotaxic injection reduced dopaminergic neuron loss after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine -induced stress, with lower MHC-I positive neurons and T cell infiltration on the knockdown side.	(78)
Zajec *et al*	2021	Brain tissues from patients who had not suffered from cerebral diseases	Integrin β4 was among the proteins found to be significantly upregulated in intracerebral microvessels, indicating its specific expression and potential role in these areas. Integrin β4 expression was found to be confined to the basal membrane at the endothelial site, particularly between endothelial cells and astrocytes, and extended upward in the cell processes of these cells, highlighting its role in the structural organization of brain vessels. Immunohistochemical analysis revealed strong expression of integrin β4 in small vessels and capillaries in the gray and	(74)

			white matter, as well as in the pia-arachnoidal surface and choroid plexus. Expression was minimal in ependymal cells.	
Ignarro *et al*	2022	Astrocytes from human neocortical samples	In human astrocytes, β2m predominantly localized in cytoplasmic vesicles, with intensified labeling in IFN-β-treated cells.	(81)

Annex III – Brief summary of the findings described in section 4.3

Authors	Year	Cells/Tissues/Organism	Results	References
Martinez *et al*	1993	Control patients Patients with Dementia of Alzheimer's Type (DAT) Patients with Multi-infarct Dementia (MID)	Cerebrospinal fluid (CSF) was significantly elevated in patients with AD versus controls but not versus control patients. The CSF-β2m / serum-β2m ratio was significantly increased in DAT patients. There was a negative correlation between CSF IL-1β concentration and CSF β2m in DAT patients, but not in MID patients.	(108)
Mogi *et al*	1995	Brains of control patients Brains of PD patients	Significantly higher β2m content in the nigro-striatal dopaminergic regions of parkinsonian brains compared to control subjects.	(103)
Carrette *el al*	2003	Control patients Patients with AD	In CSF of AD patients there was an increase in the protein expression of β2m.	(109)
Zhang *et al*	2008	Control patients Patients with AD or with mild cognitive impairment (MCI) or with frontotemporal dementia (FTD) or with PD	CSF β2m concentration significantly increased only between control and PD groups.	(104)
Doecke *et al*	2012	Healthy patients Patients with AD from the Australian Imaging Biomarker and Lifestyle (AIBL) and Alzheimer Disease Neuroimaging Initiative (ADNI) Cohorts	Plasma β2m content was reduced in AD participants compared to healthy controls.	(110)
Smith *et al*	2015	Wild type mice β2m knockout mice Humans	Concentration of β2m in plasma increased in aged (18 and 24 months) compared to young (3 months) mice. In addition, concentration of β2m measured in archived plasma and CSF samples from healthy humans between 20 and 90 years of age showed an age-related increase. Plasma derived from young heterochronic parabionts after exposure to aged blood exhibited increased β2m compared to age-matched young isochronic parabionts. Systemic administration of soluble β2m protein to young mice led to impaired learning and memory deficits and decreased the number of newly born neurons, progenitors, and proliferating cells in the dentate gyrus of mice. Stereotaxic injection of β2m into the hippocampus resulted in decreased number of newly born neurons and progenitors, both in vivo and in vitro. Age-related increase in β2m protein expression was detected in the hippocampus of both young and aged animals.	(102)

			Effects of β2m on cognitive function were reversible, as impairments in hippocampal-dependent learning and memory were no longer apparent after an extended recovery period. Inhibition of hippocampal-dependent learning and memory by exogenous β2m was dependent on surface MHC I expression, as evidenced by experiments with Tap1-knockout mice. β2m-knockout mice exhibited enhanced spatial learning and memory functions, as well as increased adult neurogenesis, neuronal differentiation and survival, particularly in aged mice.	
Yang *et al*	2017	Healthy patients Healthy elderly patients Elderly patients with age-related cognitive impairment	β2m plasma levels correlated with age in healthy groups, indicating increased β2m levels with age. There was a significant difference in plasma β2m levels between heathy advanced age and dementia group, indicating increased β2m levels in the dementia group.	(101)
Dominici *et al*	2018	Plasma samples from healthy patients Plasma samples of patients with MCI or AD	AD patients had higher plasma β2m levels compared to MCI and healthy patients.	(105)
Huo *et al*	2022	Control patients Patients with brain injury	Before and after the first Hyperbaric oxygen (HBO) therapy, blood β2m concentrations decreased significantly in brain injury patients compared to controls. Before, after the first and second courses of HBO therapy, blood β2m concentrations increased significantly in patients with conscious disturbance compared to those with non-conscious disturbance. Before HBO therapy, urine β2m concentrations were significantly higher in patients with conscious disturbance compared to those with non-conscious disturbance. In patients with conscious disturbance, urine β2m levels were correlated with Glasgow Coma Scale score grade and Coma Recovery Scale-Revised score. Blood β2m levels were correlated with Mini Mental State Exam (MMSE) score grade in patients with non-conscious disturbance. Blood β2m concentrations increased significantly between mild and moderate and mild and severe grades in the MMSE score grades. No significant difference was observed between moderate and severe grades. Both blood and urine β2m content showed significant increases in their evaluation values for assessing consciousness disorder.	(113)
Chen *et al*	2023	Sprague-Dawley wild-type mice Middle Cerebral Artery Occlusion (MCAO) mice	In MCAO mice, elevated β2m levels were detected in the serum, CSF, and brain tissue at 3 hours and 1, 3, 7, and 14 days post-MCAO. β2m expression peaked at 3 hours after MCAO and returned to normal at 3 and 14 days. β2m was primarily expressed in Neuronal Nuclear antigen-positive	(107)

		β2m RNA interference MCAO mice	neurons. MCAO mice treated with β2m interference RNA had a decrease in the number of β2m-positive cells and β2m protein levels in the ischemic penumbra of MCAO rats compared to controls. β2m knockdown in MCAO mice reduced cerebral infarct volume, cognitive deficits and glial and NLR Family Pyrin domain containing 3 activation. Hematoxylin-Eosin staining showed cell loss, nuclear condensation, and inflammatory cell infiltration in MCAO rats, which were alleviated by β2m knockdown.	
Zhao *et al*	2023	Postmortem brain tissues of aged-matched controls Postmortem brain tissues of AD patients 5×FAD transgenic mice APP/PS1 transgenic mice β2m knockout mice Tap1 knockout mice $β2m^{KI/KI}$ mice	Elevated β2m expression was observed in the cerebral cortex of individuals with AD and 5×FAD mice, correlating positively with $Aβ_{42}$ levels. β2m was predominantly expressed in the microglia of $β2m^{KI/KI}$ mice and wild-type mice treated with oligomeric $Aβ_{42}$ and LPS, NF-κB inhibitor and STAT3 inhibitor affected this upregulation. Amyloid plaques in human AD and 5×FAD mouse brains had a β2m-immunopositive core surrounded by Aβ. Adeno-Associated Virus-β2m injection in 5×FAD mice increased amyloid deposits, soluble and insoluble $Aβ_{42}$ levels, and dystrophic neurites. β2m injection increased amyloid deposition in Tap1 knockout mice. Genetic ablation of β2m in 5×FAD mice reduced amyloid deposition, soluble and insoluble $Aβ_{42}$ levels, and protected cognitive function. Genetic ablation of β2m in mice prevented Aβ-induced memory deficits and hippocampal dendritic spine loss. Antisense oligonucleotide targeting β2m reduced amyloid pathology in 5×FAD; $β2m^{KI/KI}$ mice. Anti-β2m antibodies reduced Aβ plaque load and improved memory in 5×FAD mice. Parabiosis with β2m knockout mice or intravenous injection of anti-β2m antibody in 5×FAD mice reduced amyloid pathology and improved cognitive function. Treatment of 5×FAD; $β2m^{KI/KI}$ mice treated with β2m peptides (amino acids 51-75) effectively blocked β2m-Aβ coaggregation and reduced cognitive deficits.	(106)
Huang *et al*	2023	Chinese Alzheimer's Biomarker and Lifestyle (CABLE) cohort	Aging was a significant risk factor for AD, and plasma β2m levels were found to increase with age. Plasma β2m was significantly lower in the stage 0 biomarker group compared to stages 1, 2 and suspected non-AD pathology biomarker groups. Increased plasma β2m was linked to lower scores in the	(111)

			MMSE and Montreal Cognitive Assessment (MoCA). Higher plasma β2m correlated with lower CSF $A\beta_{1-42}$ levels, but not with Total-tau or Phosphorylated-tau. Increased β2m was also linked to a drop in CSF $A\beta_{1-42}/A\beta_{1-40}$ content and increases in Total-tau/$A\beta_{1-42}$ and Phosphorylated-tau/$A\beta_{1-42}$ levels. The association between plasma β2m and CSF AD biomarkers was not affected by sex, age, education, cardiovascular factors, subjective cognitive decline, or APOE ε4 status. $A\beta_{1-42}$ had direct, indirect, and total effects on cognition, indicating that the connection of β2m with MMSE and MoCA was mediated by $A\beta_{1-42}$. Phosphorylated-tau/$A\beta_{1-42}$ mediated the relationship of plasma β2m with MMSE	
Kim *et al*	2023	Control human brain tissue AD human brain tissue SH-SY5Y cells	SH-SY5Y cells treated with Aβ oligomers showed markedly reduced MHC-I protein levels but unchanged mRNA levels. The stability of the MHC-I- β2m complex decreased upon Aβ treatment, confirmed by reduced cell surface MHC-I-β2m levels (FACS analysis) and increased free MHC-I heavy chains. Aβ treatment also increased β2m release into the medium, indicating complex dissociation. Synaptic MHC-I and β2m levels were reduced in AD brains compared to controls. Immunoprecipitation showed that most synaptic MHC-I in control brains was in the MHC-I-β2m complex form, while in AD brains it was primarily in the β2m-free form. This destabilization was not due to impaired complex formation in the ER or altered glycosylation maturation.	(112)

Annex IV – Brief summary of the findings described in section 4.4

Authors	Year	Cells/Tissues/Organism	Results	References
Wolf and Cook	1995	A.Tla^{b} mice B6.K1 mice C3H.L^{d} transgenic mice BALB/c-H-2^{dm2} mice B10.A(3R) mice C57BL/6 mice Concanavalin A-activated T cell blasts (CAB)	Both 48-kD and 50-kD Qa-1 proteins were found in B6 and BALB/c mice, but only the 48-kD protein was found in C3H mice. In some cells, the 50-kD Qa-1 protein was found together with the H-2L^{d} protein. Qa-1 and H-2L^{d} association occurred post-Golgi, after glycosylation. In B6 mice, a different 50-kD protein (Qsm) bound with Qa-1, changing how Qa-1 appeared on the cell surface.	(120)
Balendiran *et al*	1997	H-2L^{d} molecules	The H-2L^{d} structure was similar to H-2K^{b} and H-2D^{b}, but had unique interdomain interactions with β2m. H-2L^{d} had significantly fewer van der Waals interactions and H-bonds between light and heavy chains compared to H-2K^{b} and H-2D^{b}. H-2L^{d} had fewer stabilizing interactions between the a1a2 domains and β2m. The H-2L^{d} cleft was significantly more hydrophobic than H-2D^{b}.	(118)
Jelonek *et al*	1998	Soluble H-2L^{d} molecules Soluble H-2D^{d} molecules Soluble H-2K^{b} molecules Soluble CD8αα molecules Soluble CD8αβ molecules	In SPR analysis, soluble empty peptide H-2L^{d} bound strongly to surface CD8, being this effect time and concentration dependent. However, emptied soluble H-2K^{b} and H-2D^{d} molecules did not bind to surface CD8 molecules. The empty peptide H-2L^{d} molecule binding to CD8 was inhibited in the presence of peptides that are known to be ligands of H-2L^{d} (YPHFMPTNL and LSPFPFDL). The CD8α peptides 5397 and 5404 competed between each other for binding to soluble empty peptide H-2L^{d} and increased its capacity to bind to 30-5-7S monoclonal antibody. The CD8α peptide 5397 and the CD8β peptide 5405 inhibited empty peptide H-2L^{d} binding to surface CD8αβ, with varying affinities. Soluble empty peptide H-2L^{d} associated directly with the peptide 5403, which was coupled through its carboxyl terminal residue. This binding was blocked when H-2L^{d}-binding peptides were used.	(119)
Oliveira *et al*	2004	C57BL/6J wild-type mice β2m and TAP1 knockout mice	In unoperated wild-type and mutant mice, the immunoreactivity in the dorsolateral area of the motor nucleus was significantly lower compared to the ventrolateral. One week after sciatic nerve transection, there was a reduction in synaptophysin immunoreactivity in wild-type mice, but a significantly larger reduction in both β2m and TAP1 knockout	(114)

			mice. One week after sciatic nerve transection, axotomized motoneurons showed characteristic morphological changes. In β2m knockout mice, the mean synaptic cell soma covering was significantly reduced compared to wild-type mice. The absolute number of remaining synapses was lower in β2m knockout mice versus wild-type. After resection of the sciatic nerve, there were significantly fewer regenerating sciatic motoneurons were found in β2m knockout mice compared to wild-type animals.	
Sabha *et al*	2008	C57BL/6J mice A/J mice Balb/cJ mice	Basal MHC-I expression was almost absent. Post-lesion of the spinal cord, MHC-I upregulation was noted only in the ipsilateral ventral horn, varying across strains. At 1 week post-lesion, A/J mice showed a robust MHC-I increase near lesioned motoneurons. Balb/cJ and C57BL/6J showed less intense and more diffuse staining. At 3 weeks post-lesion, no statistical difference was observed among strains due to an increase in Balb/cJ and C57BL/6J. A significant decrease in synaptophysin expression occurred in the ipsilateral motor nuclei, with a greater reduction in A/J and Balb/cJ mice compared to C57BL/6J. Recovery was seen in A/J and Balb/cJ at 3 weeks, whereas C57BL/6J showed a further decrease. A/J mice showed the highest glial fibrillary acidic protein levels, followed by Balb/cJ, with C57BL/6J mice having the lowest levels at 1 week post-lesion. At 3 weeks post-lesion, A/J and Balb/cJ mice displayed significantly stronger glial fibrillary acidic protein signals than C57BL/6J mice. Post-axotomy, C57BL/6J mice showed larger preservation of synaptic inputs compared to A/J and Balb/cJ. At 3 weeks, C57BL/6J did not recover afferents as A/J and Balb/cJ mice did. A/J and Balb/cJ mice exhibited significant recovery of synaptic covering compared to C57BL/6J mice. C57BL/6J mice showed a higher number of closely grouped terminals 1-week post-lesion, which normalized by 3 weeks.	(116)
Zohar *et al*	2008	C57BL/6J mice Balb/cJ mice	Colocalization of MHC Class I and Ly49 proteins in somas and axons of 1-day-old C57BL/6J mice primary embryonic cortical neurons. Ly49 mRNA expression was detected in the cerebral cortex, hippocampus, hypothalamus, and cerebellum of C57BL/6J mice. Incubation of C57BL/6J mice neurons with anti-K^b monoclonal antibody during five-days increased neurite growth 3-fold, anti-D^b increased growth 1.5-fold. Combined treatment further increased neurite growth but was lethal in longer treatments. Anti-H-2^b specific Abs had no effect on BALB/c mice neurites. Treatment of C57BL/6J mice neurons with anti-K^b or D^b mAbs decreased neurite growth and neuron survival. Anti-Ly49 monoclonal antibody suppressed neurite growth and enhanced cell survival.	(121)

			In mice cultured neurons, anti-MHC-I Abs reduced synapsin-I-positive synapses despite increased neurite growth. Anti-Ly49 Abs increased the number and size of synapsin-I positive puncta and increased synapsin-I stain in growth cones.	
Wootla *et al*	2016	HLA-A11+ mice HLA-B27+ mice Transgenic human β2m positive (Hβ2m+) mice MHC-II deficient ($A\beta^0$) mice β2m deficient ($\beta2m^0$) mice C57BL/6 mice SJL/J mice	$A\beta^0.\beta2m^0$ mice died within 16-18 days post-infection (dpi) with Theiler's murine encephalomyelitis virus (TMEV), while $\beta2m^0$, $A\beta^0$, and C57BL/6 mice survived. $A\beta^0.\beta2m^0.H\beta2m^+.A11^+$, $A\beta^0.\beta2m^0.H\beta2m^+.B27^+$, and $A\beta^0.\beta2m^0.H\beta2m^+$ mice infected with TMEV survived past 18 dpi with minimal deficits. At 18-21 dpi, $A\beta^0.\beta2m^0$ mice showed significant gray matter neuronal injury and meningeal inflammation. In contrast, $A\beta^0.\beta2m^0.H\beta2m$+.A11+ and $A\beta^0.\beta2m^0.H\beta2m$+.B27+ mice exhibited minimal gray matter pathology. At 45 dpi, $A\beta^0.\beta2m^0.H\beta2m^+.A11^+$ and $A\beta^0.\beta2m^0.H\beta2m^+.B27^+$ mice had significantly less spinal cord demyelination compared to highly susceptible SJL/J mice. At 7 dpi, both $A\beta^0.\beta2m^0.H\beta2m^+.A11^+$ and $A\beta^0.\beta2m^0.H\beta2m^+.B27^+$ mice had similar brain pathology. At 45 dpi, $A\beta^0.\beta2m^0.H\beta2m^+.B27^+$ mice showed significant brain repair, particularly in the hippocampus and cortex, whereas $A\beta^0.\beta2m^0.H\beta2m^+.A11^+$ mice showed persistent injury. Both transgenic strains had high levels of virus RNA in the brain at 7 and 45 dpi, similar between strains.	(123)
Bombeiro *et al*	2017	C57BL6/J mouse	Astrocytes and spinal neurons of C57BL/6 mice knocked down with Small Interfering RNA for β2m had reduced expression of MHC-I molecules and glial fibrillary acidic protein and increased astrocyte shrinkage. In addition, there was a reduction in the expression of the neurotrophic factors BDNF and GDNF, as well as the pro-inflammatory cytokines TNF-α, IL-1, IL-6, IL-12, and IL-17.	(115)
Loll *et al*	2020	Peptide pGR (RRRWHRWRL) HLA-B2709 molecule HLA-B2705 molecule	Similar peptide dynamics were observed in pGR-B2705 and pGR-HLA-B2709 complexes, but different dynamics in pVIPR and viral pLMP2 complexes with these subtypes. Higher flexibility in HLA-B2705 heavy chains compared to HLA-B2709 was seen in specific residues around the binding groove. pVIPR complexes showed high flexibility in HLA-B2705 but low flexibility in HLA-B2709. Enhanced flexibility was observed for peptides and MHC-I heavy chains, with specific regions showing significant flexibility in HLA-B2705 compared to HLA-B2709. Elevated conformational plasticity in Ankylosing Spondylitis-associated subtypes like HLA-B2705 correlated with the presence of cytotoxic T lymphocytes against certain peptides. Highly flexible peptide-HLA-B27 complexes, like those in B2705, were correlated with efficient negative T cell selection in the thymus. Molecular mimicry was seen in HLA-B2705 but not in HLA-B2709.	(124)

Lin *et al*	2021	C57BL/6 and C57BL/6J wild-type mice H-2K^b and H-2D^b knockout mice H-2K^b knockout mice H-2D^b knockout mice	The absence of H-2K^b in knockout mice, but not H-2D^b in knockout mice, resulted in increased neurogenesis in the adult hippocampus. H-2K^b knockout mice showed increased proliferation and neurogenesis compared to wild-type and H-2D^b knockout mice. Local abrogation of H-2K^b with viral-mediated interference RNA in wild-type mice increased proliferation and neurogenesis, while abrogation of H-2D^b had no effect. In vitro studies showed increased Neural Small Progenitor Cell proliferation in H-2K^b knockout cells from compared to wild-type and H-2D^b knockout cells. H-2K^b knockout mice had an increased proliferation due to faster cell cycle progression. Overexpressing H-2K^b in neural stem and progenitor cells of wild-type mice decreased proliferation and neurogenesis, whereas H-2D^b overexpression had no effect. H-2K^b negatively regulated neural small progenitor cell proliferation by inhibiting fibroblast growth factor receptor 1-mediated signaling pathways in neural stem and progenitor cells derived from H-2K^b knockout mice.	(122)