Dr. Nuala MOONEY
Equipe 3, INSERM U976
HUMAN IMMUNOLOGY, PATHOPHYSIOLOGY & IMMUNOTHERAPY
Université Paris Diderot, Sorbonne Paris Cité
Hôpital Saint-Louis
Equerre Bazin
1, avenue Claude Vellefaux
75475 PARIS CEDEX 10

Tél. : 01 57 27 68 38
Mél : nuala.mooney@univ-paris-diderot.fr

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Report on the doctoral thesis of *Ms Maria Camila TOVAR FERNANDEZ*

Title: *Origin of antigenic peptides in MHC class I pathway*

Promoter: *Dr Robin FAHREUS*

A fundamental role of the immune system is to distinguish between self and non-self. This is partly achieved by the presentation of non-self antigenic peptides (eg of viral origin) by Major Histocompatibility Complex class I (MHC-I) molecules to cytotoxic $CD8^+$ T cells leading to cytolysis of the antigen presenting cell. Both cancer and viral immune evasion may therefore be mediated by impaired presentation or provision of MHC-I antigenic peptides.
The research carried-out for this doctoral thesis concerns the origin of antigenic peptides arising from two sources; protein degradation by the process of autophagy and pioneer translation products derived from pre-spliced mRNA.

The manuscript is composed of a general Introduction to the MHC, antigen processing, presentation and cross-presentation of peptides. This is followed by a more detailed Introduction (Part 1) to the Autophagy pathway. This part of the Introduction is essential in order to understand the first objective of the thesis studies. It begins with a detailed description of Autophagy and of how selective Autophagy regulates quality control, followed by a summary of the published literature concerning Autophagy and MHC-I presentation and the role of different substrates of Autophagy. Part 1 continues by outlining the aims of the research performed, the Materials and Methods used followed by a Results section and a Discussion of the results and conclusions of this part of the study.

The key results from this part of the thesis are that siRNA mediated knockdown of Atg5 and Atg7 impaired Autophagy without altering the cell surface expression of MHC-I; the exogenous SL8 peptide was presented whether or not autophagy was deficient. In contrast, when presentation of an OVA construct was tested, with or without the ability to form aggregates, inhibition of Atg5 and Atg7 reduced antigen presentation and the reduction was independent of aggregate formation. Because the proteasome has a major role in antigen processing in the canonical MHC-I antigen presenting pathway, proteasomal activity was inhibited and the presentation of OVA-derived peptides was determined. Inhibition of proteasomal activity strongly reduced presentation of OVA antigenic peptide.
The next series of experiments examined MHC-I presentation of Epstein-Barr virus -encoded EBNA1 protein derived peptides. The SL8 peptide was inserted into the EBNA1 open reading frame and aggregate promoting sequences were included in the construct or not. Inhibition of autophagy did not alter EBNA1 derived peptide presentation by the MHC-I pathway (although this is not the case for the MHC-II pathway). The lack of implication of autophagy in EBNA1 derived peptide presentation was further confirmed by chloroquinine mediated inhibition of autophagy in the same experimental model. Finally, when OVA was conjugated with a glycine-alanine repeat (GAr),sequence, OVA was no longer presented to the MHC-I pathway via autophagy.
Together the part of the thesis studies provides important information regarding the non-identical potentials of different substrates as candidates for peptide presentation implicating the pathway of Autophagy.

The second part of the thesis follows a similar format with an Introduction that is detailed regarding the source of antigenic peptides for the MHC I pathway; particular attention is paid to alternative mRNA translation as a source of peptides. The aim is outlined and the Material and Methods are detailed followed by a Results section. The main results stemming from this part of the work reveal that MHC I peptides can be derived from intron sources, that intron derived peptides could be localized and that the pre-spliced antigenic peptide sequences were within the light polysome fraction.

Throughout the thesis, the methods undertaken in order to perform studies were varied and appropriate to address the questions asked, molecular and cellular biology approaches were taken in addition to antigen presentation assays of different peptides and full-length antigens. Sophisticated contructs were specifically designed to address the role of antigenic peptides of different origins.

The results revealed by studies carried out in the course of this thesis project are of high importance in the context of MHC-I antigenic peptide presentation and in particular neopeptide identification in tumour immunology.

The literature cited throughout the manuscript is appropriate and the choice of literature referred to in both the Introduction and Discussion sections of the manuscript indicate that the doctoral candidate has developed specialized knowledge in this subject and is scientifically mature.

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Part of the results have already been published *Cellular Immunology (2022)* and Ms *TOVAR FERNANADEZ* is the first author on this publication.

I therefore would like to transmit a highly favorable review of the doctoral thesis of Ms Maria Camila TOVAR FERNANDEZ.

Nuala MOONEY

Nuala Mooney
Directeur de Recherche (DR1, CNRS)