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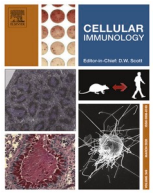

Nilabh Shastri – Towards understanding classical and non-classical MHC-I antigen processing and presentation

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ABSTRACT

Major histocompatibility complex (MHC-I) peptide antigen processing and presentation has experienced a revived interest in the context of immuno oncology, immune surveillance escape by pathogen mutations and technical advances that accelerate vaccine design. This sheds new light on the discoveries made by Nilabh Shastri and colleagues that includes the characterisation of cryptic MHC-I peptide antigen epitopes derived from untranslated regions and the *N*-terminal trimming of peptide antigen precursors by the aminopeptidase ERAAP (ERAP1/2 / ARTS1/LRAP) in the endoplasmic reticulum (ER) prior to the complete assembly of MHC-I complexes and their subsequent exposure to the cell surface. These scientific findings have important implications for developing novel therapeutic approaches in immunotherapy and modern vaccine design.

Major histocompatibility complex (MHC I & II) restriction of peptide antigens presented to *T*-lymphocytes reflects a cornerstone of the human adaptive immune response [46]. The molecular basis of MHC antigen processing and presentation has been predominantly a focus of research since more than four decades and thought to be well understood in its molecular details. Nilabh Shastri and his team uncovered some unexpected aspects of antigen processing and also the source of peptide antigens. The relevance of these discoveries has recently attracted attention in the context of neoantigen discovery in immunotherapy [22] as well as mutational effects that restrict MHC-I antigen recognition [36,23]. In particular, novel links between genetic variations in components of the antigen processing and presentation machinery as well as the source of antigens outside the protein coding region of the genome were revealed due to Nilabh Shastri's contributions.

1. Nilabh Shastri's discoveries within the MHC-I antigen processing pathway

It is in this context that highlights from contributions out of Nilabh Shastri's work are reiterated here, namely in applying elegant biochemistry, immune assays, peptide sequencing and immunopeptidomics by mass spectrometry to understand molecular aspects of antigen processing and presentation by MHC molecules. Intracellular proteins are mostly turned over by the ubiquitin protease system (UPS), which involves protein modification by poly-ubiquitin chains in a covalent manner, which serves as a recognition signal by the RPN13 subunit of the 26S proteasome complex for subsequent protein unfolding by the 19S sub-complex, deubiquitylation by POH1/USP14/UCH37 and degradation of the polypeptide chain by the core 20S proteasome catalytic complex [34,3]. A fraction of proteasomal degradation products, typically between 3 and 24 amino acids [16] is then transported by the TAP1/2 complex to the endoplasmic reticulum (ER), predominantly precursors of 9-11mer peptides optimal for binding to MHC class I molecules [42,7]. Work from Dr Shastri's team has been focused on this aspect of antigen processing, namely the involvement of HSP90 to chaperone peptides to TAP1/2 for ER translocation [19] and to examine the potential involvement of other proteolytic enzymes capable of trimming antigen precursor to the optimal length for MHC I binding in the ER [28]. Cytosolic proteases such as tripeptidyl peptidase II (TPPII) and Thimet aminopeptidase/ THOP1 were characterised to contribute [9,8,40,44]. However, due to the preference of TAP1/2 to import longer precursors into the ER, in particular *N*-terminal extensions, it became clear that additional cleaving activity existed in the ER [39,38]. Subsequent investigations revealed the discovery of ERAAP (ERAP), subsequently characterised as two enzymes [35] (ERAP1/2, also named ARTS1/LRAP), representing ER resident aminopeptidases involved in MHC-I peptide epitope precursor trimming [11,14,12] (Fig. 1). This is consistent with the fact that 26S and also 26Si (immuno)-proteasome produce predominantly correct C-terminal cleavages of MHC-I antigen epitopes, but often with *N*-terminal extensions, and precises 9-mer

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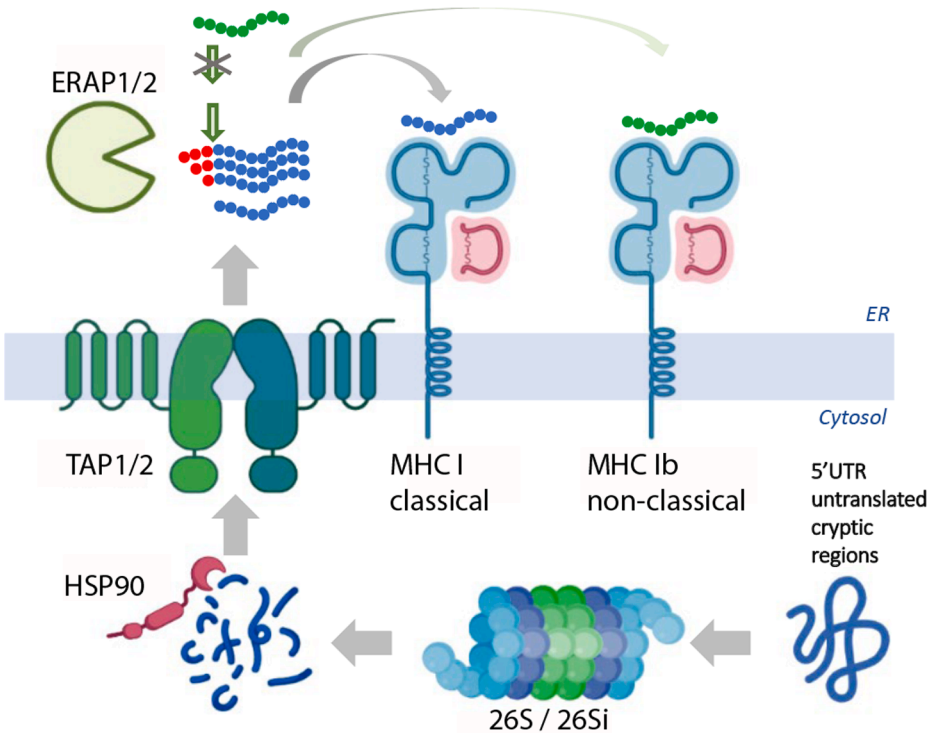

Fig. 1. Expanding the cellular antigen pool presented by classical and non-classical MHC I molecules. Major contributions from Nilabh Shastri's work revealed cellular proteins including untranslated "cryptic" regions give rise to polypeptides that are ubiquitylated and degraded by 26S/26Si proteasomes. A fraction of degradation products is chaperoned by HSP90, thereby facilitating their transport to the TAP1/2 complex for transfer to the endoplasmic reticulum (ER). ER resident aminopeptidases (ERAP1/2 / ERAAP / ARTS1/LRAP) subsequently trim peptide antigens prior to loading to MHC-I molecules in the endoplasmic reticulum (ER). ERAP inhibition affects the MHC-I peptide pool, but also favours loading of distinct peptides to MHC-Ib (Qa-1^b), thereby shaping the ligandome for classical and non-classical molecules.

generation may exist but not as the predominant product [6,17,30].

2. Contributions to technical advances in MHC peptide sequencing analysis

However, more recent technical advances in high sensitivity mass spectrometry-based immunopeptidomics revealed that *N*-terminal extended series appear to be quite commonly presented on MHC molecules on the cell surface [5,15,29,45], suggesting flexibility in MHC-I binding or, more likely, incomplete processing of C-terminally attached peptide precursors to MHC-I molecules that are subjected to further trimming. Using MS/MS-based sequencing boosted the understanding of the cellular origin of MHC-I derived peptide antigens, which confirmed earlier observation made by the Shastri team and others that a portion of them appear to be generated from untranslated regions, referred to as cryptic epitopes [21,37,41].

Also, Shastri's work revealed that the peptide antigen repertoire presented by classical MHC-I molecules was controlled by ERAAP directly, but it also affected a distinct repertoire of peptides presented by non-classical MHC class Ib (Qa-1^b) molecules [33]in a fashion that is not yet fully understood (Fig. 1) [26,27,25,10]. However, ERAAP polymorphisms.

[31,1,32] will have considerable effects on the classical as well as the non-classical MHC-I antigen repertoire. Therefore, it is expected that pharmacological intervention with ERAAP function as well as the 26S/26Si proteasome (e.g. Bortezomib - [24,43] and possibly other cytosolic proteases will have further effects on the classical and non-classical MHC-I peptide antigen repertoire in a way that has yet to be determined in detail, in particular in the clinical context.

3. Revived relevance of MHC antigen processing in immuno-oncology and vaccine design

The major molecular components in antigen processing pathways were discovered years ago, but recent advances in technical capabilities to reveal MHC-I (and II) peptide sequences at the genomic but also peptide level (mass spectrometry) revived the relevance of MHC antigen processing and presentation. This had an impact on accelerating the characterisation of neoantigens in tumours, but also on boosting the discovery of pathogen derived immunogenic antigens. Nilabh Shastri's contributions influenced discoveries of immune checkpoint inhibitor mechanisms of action, MHC cryptic epitopes and neoantigens and the relevance of mutations in ERAAP and other components of the MHC antigen processing machinery [25,10,13]. One legacy that will emerge from his work and contributions to the field is the development of ERAP1 and ERAP2 selective inhibitors that are expected to enter the clinic for MHC-I neoepitope modulation in the context of immuno-oncology therapy [18,4,20]. Nilabh Shastri's influence has been widely recognised [13,2], and he will be greatly missed as a college and immunologist.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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