

P017 Impact of intermediate-type proteasomes in viral and tumor antigen presentation
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Less is known about differences in the proteolytic machinery involved in tumor and viral antigen generation and presentation. We selected two HLA-A*0201-restricted epitopes, MART-1₂₆₋₃₅ as a representative for melanoma and pp65₄₉₅₋₅₀₃ for HCMV for further analysis. Besides differences in antigen presentation by standard and immunoproteasomes the role of intermediate type proteasomes was examined. To analyze the catalytic properties of various intermediate-type proteasomes, we generated HeLa cells that constitutively express the immunosubunits $\beta 1i$, $\beta 2i$ and $\beta 5i$ in all possible combinations. Furthermore we examined the influence of structural changes within the proteasome complex on the catalytic activities after incorporation of the immunosubunits or catalytic inactive T1A mutants. In agreement with published data, MART-1₂₆₋₃₅ antigen presentation was not affected in cells either expressing the $\beta 5i$ -subunit or standard proteasomes. In contrast, cells with other compositions of intermediate-type proteasomes show a diminished CTL recognition comparable to immunoproteasome containing cells. Experiments with T1A mutants suggest that the catalytic activities of $\beta 5/\beta 5i$, $\beta 2$ and $\beta 1$ are necessary for the efficient presentation of MART-1₂₆₋₃₅. In contrast, cells expressing immunoproteasomes presented the pp65₄₉₅₋₅₀₃ epitope more efficiently. *In vitro* digestion experiments with purified proteasomes will show the impact of various proteasome subtypes in MART-1₂₆₋₃₅ and pp65₄₉₅₋₅₀₃ antigen presentation in more detail.