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LRA TREATMENT ALTERS ANTIGEN PROCESSING AND PEPTIDE PRESENTATION IN CD4 T CELLS.

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HIV persists as a latent infection in memory CD4 T cells. Current approaches for eradication rely on a shock and kill strategy where pharmaceutical agents reactivate the latent reservoir leading to cytopathic effects or immune clearance. Immune recognition requires the presentation of HIV peptides by MHC at the cell surface following LRA-induced HIV protein expression and processing. Some of the LRA that showed promising in vitro activity but limited efficacy in clinical trials such as HDAC inhibitors and PKC agonists have not been evaluated for their effects on HIV antigen processing and presentation by MHC.
We assessed the hydrolytic activities of cytosolic peptidases in live primary CD4 T cells treated with HDACi or PKCa using a fluorometric assay. The effects on antigen processing were studied by an in vitro degradation experiment of synthetic HIV peptides in cytosolic extracts of mock- or LRA-treated CD4 T cells. Finally, we analyzed the effects of LRA treatment of PBMCs on the MHC-peptidome by direct acid elution from the cell surface and mass spectrometry analysis.
Treatment with HDACi significantly decreased the hydrolytic activities of the proteasome and cytosolic aminopeptidases in primary CD4 T cells by up to 0.8-fold, whereas treatment with PKCa increased these activities by up to 4.9-fold. Consequently, the degradation of HIV synthetic peptides in cytosolic extracts from LRA-treated primary CD4 T cells yielded altered degradation patterns with 4-8 sites significantly changed in a 35-mer and 2-4 in a 24-mer depending on the LRA. Additionally, there were changes in the generation of known epitopes as well as other potential HLA-binding peptides. Treatment with LRA partly changed the MHC-peptidome of PBMCs, showing common peptides as well as different numbers and location of peptides derived from some common source proteins.
The observed changes in the hydrolytic activities of cytosolic peptidases in primary CD4 T cells upon LRA-treatment result in altered peptide degradation and a modified MHC-peptidome. The effects are both drug- and sequence-dependent. These results suggest that productively infected and LRA-reactivated CD4 T cells might present different HIV epitopes to the immune system, thus requiring different CD8 T cells responses for effective clearance of reservoirs.

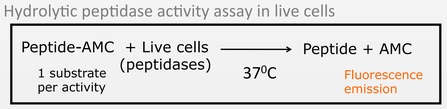
❖ 5 LRA selected: *in vitro* effect, clinical trial done or ongoing
- Panobinostat 30nM (HDAC inhibitor)
- Romidepsin 40nM (HDAC inhibitor)
- Disulfiram 500nM (Phosphatase and tensin homolog inhibitor)
- Bryostatin-1 10nM (PKC agonist)
- Ingenol-3-hexanoate 50nM (PKC activator)

❖ Cellular activation through aCD3/28 beads (similar results obtained with PHA)

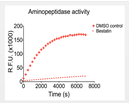
❖ 3 IMA selected: *in vitro* effect
- Ruxolitinib 100nM (Janus kinase inhibitor)
- Rapamycin 100nM (mTOR inhibitor)
- Cyclosporin A 100nM (calcineurin inhibitor)

❖ Effects of aCD3/28 and LRA-treatment on peptidase activities and surface activation marker expression

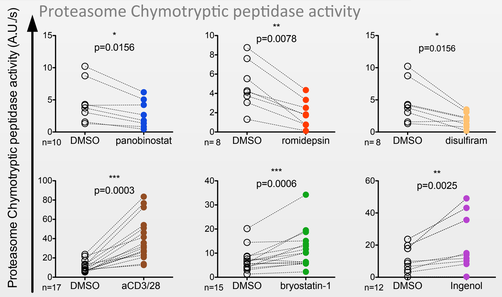
Hydrolytic peptidase activity assay in live cells



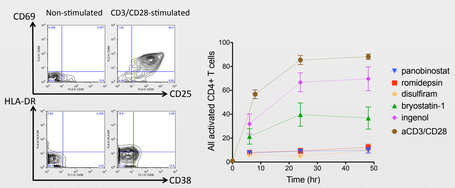
Peptidase/specific inhibitor:
- Aminopeptidase/Bestatin
- Chymotryptic/Mg132
- Tryptic-like/Mg132
- Caspase-like/Mg132



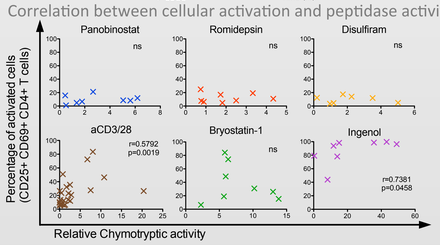
Proteasome Chymotryptic peptidase activity



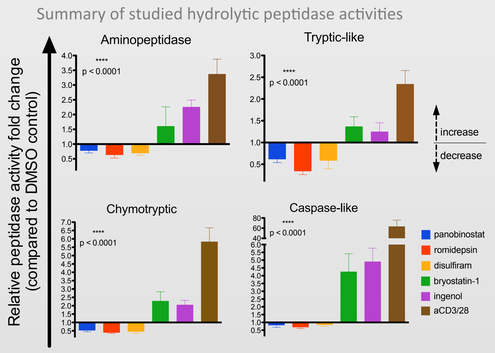
Cell surface expression of activation markers



Correlation between cellular activation and peptidase activity



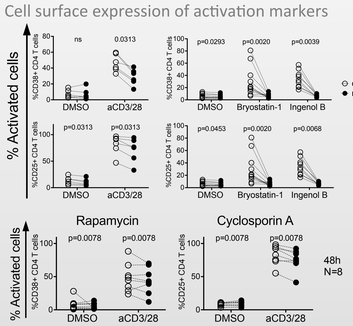
Summary of studied hydrolytic peptidase activities



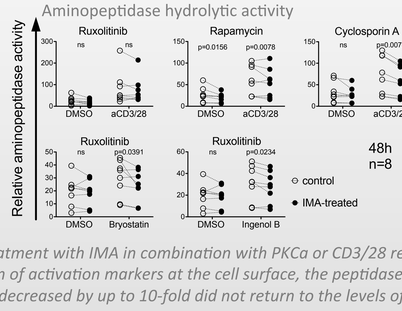
The changes in peptidase activities are drug-dependent. Similarly to stimulation with aCD3/28, the increase in peptidase activities observed upon ingenol-treatment is positively correlated with the percentage of activated cells present in the sample.

❖ Effects of IMA-treatment in combination with aCD3/28 or LRA on peptidase activities and cellular activation

Cell surface expression of activation markers



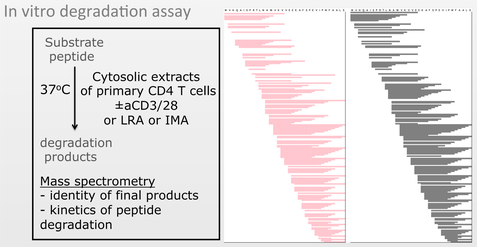
Aminopeptidase hydrolytic activity



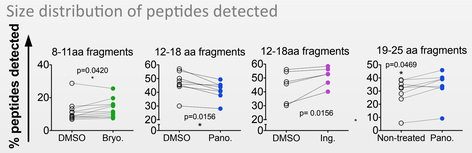
While treatment with IMA in combination with PKCa or CD3/28 reduced the expression of activation markers at the cell surface, the peptidase activities although decreased by up to 10-fold did not return to the levels of mock-treated cells.

❖ Degradation patterns of HIV-1 Gag p24-10-35m peptide are changed by aCD3/CD28-stimulation, LRA- or IMA-treatment

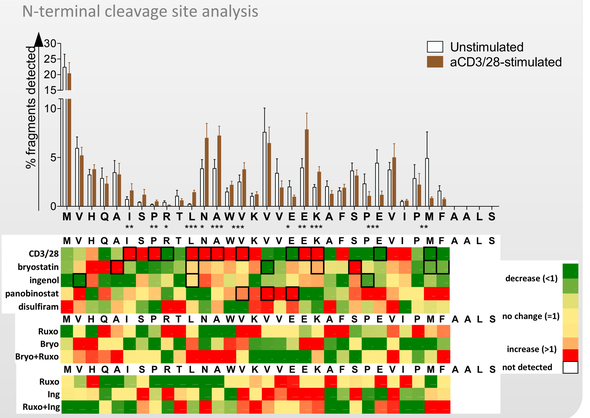
In vitro degradation assay



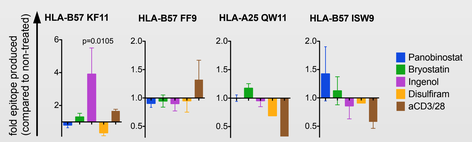
Size distribution of peptides detected



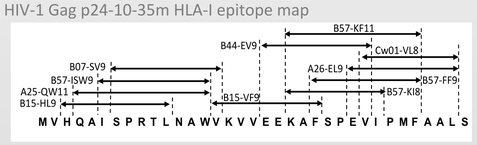
N-terminal cleavage site analysis



Optimal CD8 epitope production



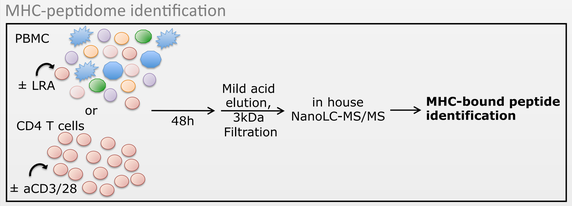
HIV-1 Gag p24-10-35m HLA-I epitope map



The changes in degradation patterns, epitopes production and cleavage sites are drug-, sequence- and donor-dependent. Combination treatment with IMA does not reverse the effects of Ingenol or Bryostatin-1.

❖ Treatment with aCD3/28 or LRA alters the MHC-peptidome of primary cells

MHC-peptidome identification



Peptide maps

For a given source protein, the identified MHC-bound peptides are mapped to the sequence and color coded per each donor. Peptides common to the treated and non-treated samples of an experimental set (=1 donor) are bolded and highlighted in grey.

common peptide
unique peptide

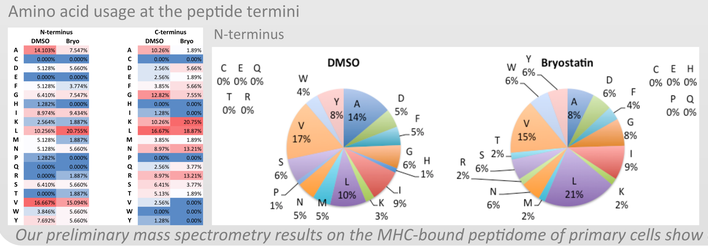
P14174 Macrophage migration inhibitory factor MIF



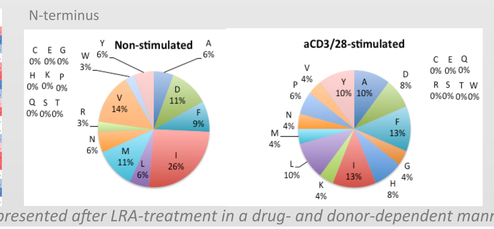
P04406 – Glyceraldehyde-3-phosphate dehydrogenase GAPDH



Amino acid usage at the peptide termini



N-terminus



Our preliminary mass spectrometry results on the MHC-bound peptidome of primary cells show differences in the peptides presented after LRA-treatment in a drug- and donor-dependent manner.

The observed changes in the hydrolytic activities of cytosolic peptidases upon LRA-treatment result in altered peptide degradation and a modified MHC-peptidome in primary CD4 T cells. The effects are both drug- and sequence-dependent. The increase in peptidase activities following Ingenol treatment or aCD3/28-stimulation is positively correlated with an the percentage of activated cells present in the sample. The use of immunomodulatory agents, while decreasing cellular activation, did not restore the peptidase activities and peptide degradation patterns to those observed for untreated cells. The modulation in antigen processing and peptide presentation caused by drug treatment diversifies the range of CD8 responses needed for clearance of reactivated HIV-infected cells; productively infected and LRA-reactivated CD4 T cells might present different HIV epitopes to the immune system, thus requiring different CD8 T cells responses for effective clearance of reservoirs.

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