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Identifying Immunogenic CD4+ T-cell Epitopes of Myeloid Cell Leukemia 1 Using Overlapping 20-mer Peptides Spanning the Whole Protein

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Introduction

Myeloid cell leukemia 1 (Mcl-1) is an anti-apoptotic protein which is overexpressed in various leukemia and other cancers [1]. Mcl-1 has a very short half-life [2], which has been suggested as a molecular mechanism for cells to switch into either the survival or apoptotic pathways in response to different stresses [3]. Recently, it has been demonstrated that downregulation of Mcl-1 by various pharmacological agents or genetic approaches dramatically increases ABT-737 lethality in various malignant cell types [4]. Different strategies for targeting Mcl-1 include (i) small interfering RNA [5] (ii) small-molecule inhibitors [6] and (iii) peptide inhibitors [7]. In recent years, therapeutic vaccination with synthetic peptides derived from anti-apoptotic proteins such as Mcl-1 has emerged as a promising strategy against hematological cancers.

In this study, 34 overlapping 20-mer peptides, spanning the entire Mcl-1 protein, were adjuvanted with cationic liposomes [8] and tested in three different mouse strains with varied Major Histocompatibility Complex (MHC) haplotypes (FVB [H-2q], CB6F1[H-2b/d], B6CBAF1 [H-2b/k]) to identify immunogenic CD4+ T-cell epitopes.

Results and Discussion

The peptides were synthesized by Fmoc SPPS on a TentaGel S Ram Resin. Each amino acid was coupled in 3-fold excess using HATU, HOAt and DIEA (1:1:1.5) in DMF. Deprotection of the Fmoc group was effected by 20% piperidine in DMF. Following synthesis the peptides were cleaved from the resin for 2h using reagent TFA/TIS/DTT/H_2O (88:2:5:5). The products were then purified by preparative RP-HPLC and characterized by MALDI-TOF-MS.

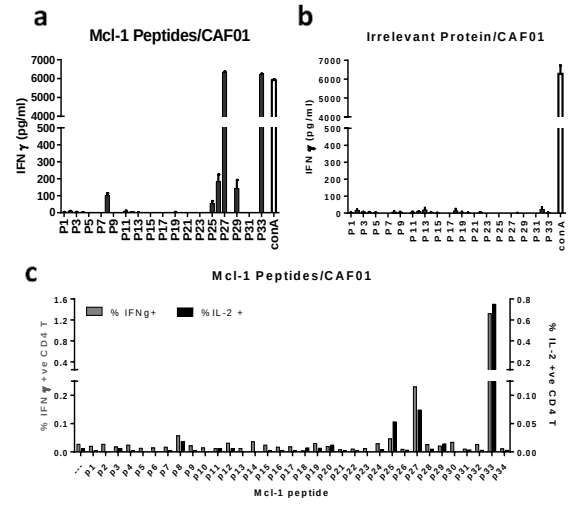

Fig. 1. Immunogenicity of Mcl-1 peptides in CB6F1 mice.

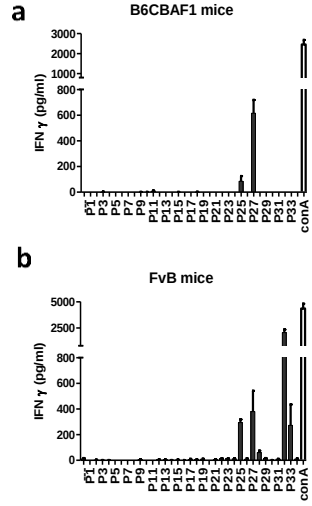

Fig. 2. Mcl-1 peptides in B6CBAF1 and FvB mice.

MFGLKRNAVI	GLNLYCGGAG	LGAGSGGATR	PGGRLLATEK	EASARREIGG
GEAGAVIGGS	AGASPPSTLT	PDSRRVARPP	PIGAEVPDVT	ATPARLLFFA
PTRRAAPLEE	MEAPAADAIM	SPEEELDGYE	PEPLGKRPAV	LPLLELVGES
GNNTSTDGSL	PSTPPPAEEE	EDDLYRQSLE	IISRYLREQA	TGAKDTKPMG
RSGATSRKAL	ETLRRVGDGV	QRNHETAFQG	MLRKLDIKNE	DDVKSLSRVM
IHVFSDGVTN	WGRIVTLISF	GAFVAKHLKT	INQESCIEPL	AESITDVLVR
TKRDWLVKQR	GWDGFVEFFH	VEDLEGGIRN	VLLAFAGVAG	VGAGLAYLIR

Mcl_1: MFGLKRNAVI GLNLYCGGAG; Mcl_2: GLNLYCGGAG LGAGSGGATR etc.

Fig. 3. Mcl-1 Sequence.

To investigate the induction of CD4 T cell responses following Mcl-1 peptide vaccination, mice were immunized s.c. with a pool of overlapping peptides spanning the human Mcl-1 sequence adjuvanted with cationic liposomes (CAF01). CAF01 is known to specifically drive a strong CD4+ Th1/Th17 response [9-10]. Splenocytes from immunized mice were restimulated with individual Mcl-1 peptides and IFNγ released measured after three days to evaluate peptide immunogenicity.

Initial immunizations were focused on responses in B6 x BALB/c F1 (CB6F1) mice carrying the H-2b and H-2d MHC haplotypes. Compared to mice immunized with an irrelevant protein, Mcl-1 peptide immunized mice generate distinct immune responses to several peptides, including weak responses to Mcl_8, 25, 26 and 29 and dominant responses to Mcl_27 and 33 (Figure 1a,b). Intracellular cytokine staining confirmed that these were CD4 (but not CD8) T cell responses, capable of producing IFNγ, IL-2 and TNFα (Figure 1c and data not shown). Immunization of B6 x CBA F1 (B6CBAF1, H-2b/k) mice with Mcl-1 peptides generated a weak Mcl_25- and a strong Mcl_27-specific CD4 T cell response (Figure 2a). These responses were also both seen in CB6F1 mice, suggesting they are H-2b restricted. Notably, although several additional peptides were predicted to contain H-2k binding peptides, none of these were found to be empirically immunogenic in our experiments. In FvB (H-2q) mice, Mcl_32 was uniquely immunogenic (Figure 2b), while Mcl_25 and Mcl_27 peptides displayed MHC haplotype immunogenic promiscuity.

In conclusion, we found that the ensemble of overlapping Mcl-1 peptides (Figure 3) contained many immunogenic MHC II-restricted peptides. Future experiments to confirm that these CD4+ T cells induced against novel epitopes can recognize Mcl-1 over-expressing cells and enhance anti-tumor immunity *in vivo* will be important to determine whether such responses are indeed effective in tumor control.

Acknowledgments

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