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N-terminal functional region of the invariant chain efficiently targets the binding of a CTL epitope to MHC class I molecules during cross-presentation

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ABSTRACT. Cross-presentation (CP) is important for priming T cell responses to many viral, bacterial, and tumor antigens. Here, we designed two Ii mutants, based on evidence that the invariant chain (Ii, also named CD74) binds newly synthesized MHC class I molecules with the class II-associated invariant chain peptide (CLIP) region of Ii, which occupies the peptide-binding groove. Specifically, we designed (1) Ii-O257, which is a CLIP-substituted Ii chimer, in which OVA257-264 (SIINFEKL) was substituted for CLIP, and (2) Ii-, also named CT257, which is a C-terminal truncated form of Ii-O257 that contains the N-terminal flanking region of Ii. We immunized C57BL/6 mice with these recombinant proteins. Real-time PCR detected that mice immunized with either Ii-O257 or Ii-CT257 recombinant proteins exhibited increased IFN-γ mRNA expression (approximately 11-fold and 13-fold, respectively) and increased IL-2 mRNA expression (approximately 9-fold and 11-fold, respectively), compared to mice immunized with the OVA257-264 peptide. *In vivo* cytokine analysis

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showed that recombinant Ii proteins were highly efficient at activating T cells. Confocal microscopy and co-immunoprecipitation showed that the 2 Ii-OVA257-264 chimers are associated intracellularly with H-2K^{b} molecules. Thus, Ii-CT257 (amino acids 1-89) binds stably to MHC class I with high affinity, indicating that it is a minimal functional fragment of the Ii immune vector. In conclusion, the N-terminal functional region of the Ii fusion protein containing CTL epitopes might prove to be useful for developing peptide or DNA vaccines that use CP as the main mechanism for CD8^{+} T cell stimulation.

Key words: Cross-presentation; MHC class I; Invariant chain; CTL epitope; RT-PCR; Colocalization

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