PREDICTION OF T-HELPER EPITOPE DOMINANCE ON THE BASIS OF CONFORMATIONAL STABILITY IN THE V ANTIGEN OF YERSINIA PESTIS

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Previous studies have shown that antigen structure directs CD4+ T-cell epitope dominance. Immunodominant regions are found flanking regions characterized by low conformational stability.

The purpose of these studies was to test the hypothesis that immunodominant epitopes can be accurately predicted on the basis of three dimensional structure in the V antigen of Yersina pestis, the causative agent of the black plague. Five immunodominant regions and several subdominant regions were predicted to lie between conformationally unstable segments identified by high crystallographic B factors, high solvent-accessible surface, low COREX residue stability, and high sequence entropy.

Groups of C57BL/6 and CBA mice were immunized intranasally with a recombinant subunit vaccine, F1-V, in which V antigen is fused to the C-terminus of the capsular region protein F1. The vaccine was administered in combination with mutant (R192G) heat-labile toxin as adjuvant. Epitope maps were determined by restimulation of splenocytes with a series of peptides spanning the length of V antigen, followed by measurement of tritiated thymidine incorporation in order to determine cell proliferation.

Combining epitope-mapping data from both strains of mice, the analysis revealed six immunodominant segments. Three of the experimentally-observed immunodominant segments coincided with predicted immunodominant segments, and two others coincided with regions predicted to be subdominant. Two predicted immunodominant segments were not observed, and one experimentally-observed segment was not predicted.

Further studies will examine sources of inaccuracy in the prediction. The failure to observe predicted epitopes could be due to insensitivity of the assay based on cell proliferation. Some epitopes may be associated with cytokine secretion and not cell proliferation. The supernatants of the restimulated splenocytes will be tested for the presence of IFNg, IL-2, and IL-4 in an effort to identify epitopes that were not reported by cell proliferation.

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