EPITOPIC MAPPING OF TWO ANTI-HUMAN INTERLEUKIN-2 MONOCLONAL ANTIBODIES USING A PHAGE DISPLAYED-PEPTIDE LIBRARY

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Introduction

Phage displayed-peptide libraries appear to be powerful tools to isolate peptide sequences binding to target molecules. IL-2 has been defined as a class of phylogenetically well-conserved molecule that plays a pivotal role in the immune response regulation. Monoclonal antibodies (MAbs) are central to many areas of current IL-2 /IL-2R research (1). As an integral part of such structure-function studies we are currently cataloguing the binding site locations and detailed specificities of anti-IL-2 MAbs thus maximizing the information available from their use. In order to identify the epitopes recognized by two MAbs (CB-IL2.1 and CB-IL2.2) raised against human Interleukin-2, obtained in our lab, we used a peptide library consisting of nine aminoacids inserted at the N-terminal region of the f1 phage major coat protein pVIII (pVIII-9aa) (2).

Materials and Methods

Selection of the pVIII-9aa library was performed using the biopanning technique essentially as described by Felici *et al.* (2). The nucleotide sequences of the gene VIII inserts were determined by single stranded DNA dideoxy sequencing with the chain termination method.

Results and Discussion

The aminoacid sequences of the inserts were deduced from the nucleotide sequences of the amino terminus of pVIII, and aligned according to their sequence similarity. The epitope recognized by MAb CB-IL2.1 is located in the loop region connecting helix B' with helix C, and involves the beginning of the helix C as well. Among the aminoacids corresponding to the consensus motif L_{72}, S_{75}, K_{76}, L_{80}, R_{81} and R_{83} are highly exposed to the solvent.

The continuous epitope sequence recognized by MAb CB-IL2.2 was initially identified by the presence of homology to the T_{101}, T_{102}, F_{103}, M_{104}, motif found at the opposite site of the four helix bundle, in a loop that connects helix C with the beta strand Y_{107}-A_{112} (see Figure).

MAbs directed against native proteins are useful tools for structural and functional studies. Defining the key residue requirement for antibody binding has therefore important implications in the elucidation of relationships between protein structure and function.

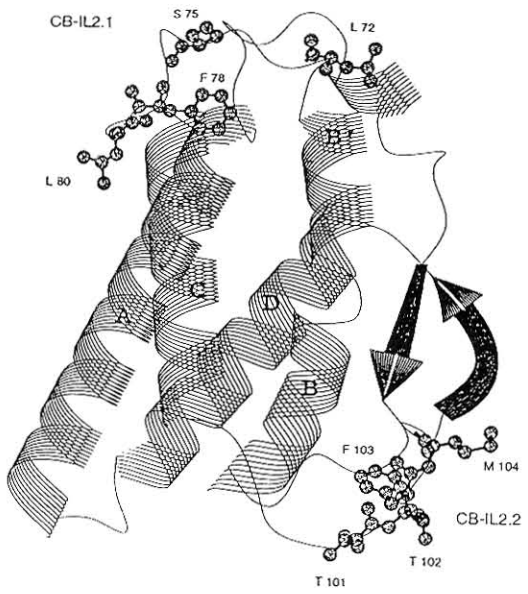

Figure. Ribbon representation of the structure of the human Interleukin-2. The side chains corresponding to the most conserved aminoacids in the mimotope sequences are highlighted. MAb CB-IL2.1 recognized the region between aa 70 and 83 and MAb CB-IL2.2 recognized the region between aa 101 and 104. Graphical representation was performed with the WHATIF program.

Trabajos seleccionados del Congreso Biotecnología Habana '97, diciembre 1-6 1997

Selected papers from Congress Biotecnología Habana '97, December 1-6 1997.

1. Moreau JL, Bossus M, Degroote D, Francois C, Jacques Y, Tartar A *et al.*: Characterization of a monoclonal antibody directed against the NH2 terminal area of interleukin-2 (IL-2) and inhibiting specifically the binding of IL-2 to IL-2 receptor beta chain (IL-2R beta). Molecular Immunology 1995;32:1047-1056.

2. Felici F, Castagnoli L, Musacchio A, Jappelli R, Cesareni G. Selection of Antibody Ligands from a Large Library of Oligopeptides Expressed on a Multivalent Exposition Vector. J Mol Biol 1991;222: 301-310.