Immunological properties of an N-terminal fragment of herpes simplex virus type 1 glycoprotein D expressed in Escherichia coli

Brief Report

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Summary. The N-terminal fragment, comprising residues −5 to 55 of herpes simplex virus type 1 glycoprotein D was expressed as a β-galactosidase fusion protein in Escherichia coli. This gD-fusion protein reacts with monoclonal antibody LP 14 directed against glycoprotein D of HSV. Antisera obtained after immunization of rabbits with purified gD-fusion protein react with HSV-1 gD in a Western blot and with N-terminal synthetic peptides of gD. In addition, these antisera are able to neutralize viral infectivity in vitro.
Mabs reactive with gD. They specified a minimum of 6 distinct epitopes that can be grouped into 4 antigenic sites. Two of these sites (I and II, represented by a single epitope and 3 different epitopes, respectively) are compatible with groups I and II, respectively [9]. The two other antigenic sites (designated as IX and X, represented by a single epitope and one or more epitopes, respectively) do not belong to any existing group, indicating that a complete definition of the antigenic structure of gD is still lacking.

To investigate the antigenic properties of a large N-terminal fragment of gD, a polypeptide containing amino acid residues -5 to 55 of gD was produced in bacteria by recombinant DNA techniques. This fragment, expressed as a cro-lacI-lacZ fusion protein, is larger than any of the synthetic gD-peptides investigated, so far [2, 5, 8, 19, 28].

To construct the plasmid for expression of the sequence -5 to 55 of gD, DNA was isolated [26] from Vero cells infected with HSV-1 McIntyre. The cloning procedures were according to Maniatis [17]. The N-terminal coding section of gD was isolated by successive cloning of a 4.4 kb HindIII/BamHI HSV-1 DNA fragment, comprising the entire gD gene [18], and of a 282-bp Sau 3A subfragment, comprising the signal peptide and the 55 N-terminal amino acids of gD. This fragment was purified by size-exclusion high-performance liquid chromatography (HPLC) on a TSK 4000 SW column (600 x 7.5 mm, Toyo Soda, Tokyo, Japan) and cloned as a pBR 322 hybrid in E. coli. For the subsequent cloning in the expression vector pEX 3 [24], the 175-bp Sau 3A-NcoI fragment was purified by gel electrophoresis, made blunt-end and ligated into the SmaI site of pEX3. This final construct (see Fig. 1) pEX3/gD_5_55, codes for the fusion protein cro-lacI-lacZ-gD_5_55, which is expressed under the