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

Brief Report

C. H. M. Kocken¹, H. J. Geerligs¹, C. A. Bos¹, G. AB², W. J. Weijer¹, J. W. Drijfhout¹, G. W. Welling¹, and S. Welling-Wester¹

¹ Laboratorium voor Medische Microbiologie, Rijksuniversiteit Groningen
² Biochemisch Laboratorium, Groningen, The Netherlands

Accepted October 3, 1988

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.

Glycoprotein D (gD) of herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) is a component of the viral envelope [23]. Immunity against gD, obtained either by transfer of gD-specific monoclonal antibodies (Mabs) [1, 22] or by immunization [3, 4, 7, 15, 16, 21, 27] with gD, provides protection against HSV-infections. Initially, Eisenberg et al. [9] arranged Mabs directed against gD of HSV-1 into 7 groups, according to various biological properties. Three of these groups (II, V, and VII) are directed against continuous epitopes and the sequences involved, residues 268–287, 340–356, and 11–19, respectively, have been identified by the reaction of the Mabs with synthetic peptides covering gD sequences [5, 10]. The remaining groups of Mabs (I, III, IV, and VI) recognized discontinuous epitopes of gD [6]. Minson et al. [19] isolated Mabs LP 14, AP 7, AP 12, and LP 2 directed against gD. Mutants of HSV resistant to these Mabs have been sequenced, and the nucleotide changes conferring resistance have been localized. Mutations in gD occurred at residues 16, 25, 129, and 216, respectively. Of these sites, residue 16 lies within the group VII epitope. Recently, Highlander et al. [13] isolated a new panel of neutralizing
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 × 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 pEX3 [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

\[
\text{HSGVRGKALADSLKMPNRFGRKDLPLDQLTDPGPVRRYHIQAGLPDFQPPSLPI}
\]

---

**Fig. 1.** Schematic representation of the gD-fusion protein and the relevant amino acid sequence of gD. The gD part of the fusion protein (■), comprising residues −5 to 55, is fused to the C-terminus of the cro-lacI-lacZ sequence in the linker sequence of pEX3. Synthetic peptides of gD used to investigate the reactivity of the anti-gD-fusion protein antisera are indicated. Peptides 1-13 and 1-14 contain an additional glycine at the N-terminus as spacer. In peptides 1-13, 1-14, 9-21, and 10-24, residue 11 (methionine) is substituted by norleucine.