Serologically defined linear epitopes in the E2 envelope glycoprotein of Semliki Forest virus

Brief Report

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Summary. A set of 41 overlapping peptides, representing the complete sequence of SFV-E2 protein were synthesized and analyzed in the ELISA test against murine anti-SFV sera. No single peptide was recognized by all antisera. Eight peptides were found to be highly reactive with hyperimmune anti-SFV sera. Six out of the eight peptide sequences coincide with the most hydrophilic regions of SFV-E2. Out of these, four peptides (amino acid positions 16–35, 61–80, 166–185, 286–305) that contain the least number of alphavirus conserved residues were selected. This panel constitutes the minimal number of peptides necessary and sufficient for specific recognition of hyperimmune mouse anti-SFV sera.

Utility of site directed serology based on synthetic peptides for delineation of the target of the anti viral humoral immune response, has been demonstrated in several systems, e.g., AIDS [1–3], EBV [4], IgA response to human papilloma virus encoded nuclear antigen [5], RSV [6], and hepatitis B associated hepatocellular carcinoma [7]. Semliki Forest virus (SFV) is a member of the family Alphavirus which are important human or animal pathogens, differing in host range but similar in molecular architecture and pattern of events involved in viral replication [8]. The primary target of the SFV neutralizing immune response is E2 [9], a structural glycoprotein 422 amino acid long, the sequence of which was inferred from the known nucleotide sequence [10].

The aim of this study was to evaluate the humoral response to linear epitopes on the E2 sequence and to extend our understanding of the immunogenicity...
of defined regions on E2 envelope proteins of alphaviruses [11–13]. Two alternative approaches for the delineation of the antigenic structure of SFV-E2 were evaluated: synthesis of selected peptides based on the hydrophobic pattern of E2 protein or alternatively systematic synthesis of overlapping peptides representing the complete sequence of E2. Following the first approach, hydrophilic regions in SFV-E2, unique to SFV and flanked by conserved residues were identified [12] by overlapping the hydrophobic plots and amino acid sequences of E2 proteins of several members of the alphavirus family [12]. These 13 regions (cassettes) designated A to N (Fig. 1), were originally selected as candidates for synthetic vaccines against SFV (produced in E. coli as fused proteins [12]). In order to evaluate the potential antigenicity of each individual cassette, the 13 peptides were chemically synthesized according to Merrifield [14, 15] cleaved and deblocked employing liquid hydrogen fluoride [14, 15], and quantitated by amino acid analysis employing pre-column derivatization with PITC [16]. The individual peptides were evaluated by ELISA (see details below) with hyperimmune mouse anti-SFV ascitic fluids (AF). ICR outbred mice were immunized by i.p. injections of formalin inactivated virus $10^9$ pfu/mouse in complete Freund's adjuvant. Following three monthly injections, AFs induced by Krebs ascitic cells were collected and each AF was evaluated for its titer against the virus by ELISA [12] and its reactivity towards SFV structural proteins by Western blots [12].

A panel of fourteen hyperimmune AFs were tested against the 13 peptide cassettes by ELISA. Percent of AFs recognized by individual peptides, varied between 0% to 85%. No single peptide (or a combination of peptides) was recognized by all AFs tested. Since this approach did not result in the identification of immunodominant epitope(s) and the 13 cassettes represent less than 50% of the E2 sequence (Fig. 1), we have applied the systematic approach of mapping the continuous epitopes of a protein for which an amino acid sequence is known, via synthesis of overlapping peptides representing the complete SFV-E2 sequence. A set of 28 overlapping peptides (20 amino acid long, with overlaps of 5 amino acids) (Fig. 1) representing the complete sequence of SFV-E2 protein were synthesized employing the multiple peptide synthesis method developed by Houghten [17]. Peptides were cleaved from the resin and deblocked in a multiple vessel apparatus employing liquid hydrogen fluoride [18], and quantitated by amino acid analysis [16]. ELISA test was carried out in 96 well microtiter Polystyrene plates (Dynatech Labs, Inc.). Each well was coated overnight with 10 microgram of peptide (in 50 microliter of sodium bicarbonate buffer containing 0.6 M NaCl [19]) and blocked with 1% BSA (w/v) in PBS for 1 h at 37°C. Plates were washed three times in PBS Tween-20 0.05% (v/v) and incubated for 1 h at 37°C with 50 microtiter of the AF tested [diluted 1:100 in PBS-Tween containing 0.5% BSA (w/v)]. Rabbit anti-mouse IgG labeled with Alkaline Phosphatase (Sigma) was added to washed plates and incubated for 1 h at 37°C. Plates were washed and incubated for 30 min with the phosphatase substrate 104 (Sigma). OD was recorded at 405 nm, in a mi-