A synthetic peptide elicits antibodies reactive with the external glycoprotein of human T cell lymphotropic virus type I

Renu B. Lal, Donna L. Rudolph, Thomas J. Palker, John E. Coligan and Thomas M. Folks

1Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, Georgia 30333, 2Division of Rheumatology and Immunology, Duke University Medical Center, Durham, North Carolina 27710 and 3Biological Resources Branch, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, U.S.A.

A synthetic peptide derived from the external glycoprotein of human T cell lymphotropic virus type I (HTLV-I) (Env-5; amino acids 242 to 256) reacted with IgG antibodies in serum specimens from HTLV-I-infected individuals. C-terminal residues of Env-5 were crucial for the antibody reactivity. Polyclonal rabbit antibodies to Env-5 did not inhibit syncytium formation but such antibodies reacted specifically with gp68	extsuperscript{env} and gp46	extsuperscript{env} glycoproteins of HTLV-I in an immunoblot analysis. Immunoprecipitation of the surface-labelled MT-2 (HTLV-I-infected) cell line with anti-Env-5 precipitated the gp68	extsuperscript{env} precursor protein. It was concluded that peptide Env-5 mimics a surface-exposed epitope on the HTLV-I external glycoprotein.

Studies of human T cell lymphotropic virus type I (HTLV-I) and type II (HTLV-II) have shown a common morphology, mode of replication and genomic structure (Rosenblatt et al., 1988). The envelope gene of both viruses encodes a precursor protein (gp68) which is processed into an external glycoprotein (gp46) and transmembrane protein (gp21) (Haseltine et al., 1985).

Both gp46	extsuperscript{env} and gp21	extsuperscript{env} have several biological activities including cellular proliferation (Gazzolo & Duc-Dodon, 1987), syncytium formation (Clapham et al., 1984; Hoshino et al., 1983) and immune suppression (Ruegg et al., 1989). However, the precise envelope domains exposed on the surface and involved in HTLV receptor binding and syncytium formation are as yet unknown.

One strategy for the analysis of host immunological responses to natural HTLV infection has been to utilize synthetic peptides derived from HTLV sequences (Horal et al., 1990; Kuroda et al., 1990; Lal et al., 1991a, b). Analyses with such synthetic reagents have identified dominant viral epitopes recognized by host antibodies in response to natural HTLV infection (Lal et al., 1991a; Palker et al., 1989). For vaccine development, it is important to determine whether synthetic peptides can induce antibody responses reactive with native viral proteins. We have previously studied B cell responses to different HTLV antigens and identified an immunodominant epitope (termed Env-5) located on gp46 encompassing amino acid residues 242 to 256 of HTLV-I (Lal et al., 1991a). The purpose of this study was to characterize the Env-5 epitope further and to determine reactivity patterns of antisera raised against the Env-5 peptide.

The Env-5 peptide derived from the central region of the gp46 envelope protein (Seiki et al., 1983) of HTLV-I (position 242 to 256; Ser-Pro-Asn-Val-Ser-Val-Pro-Ser-Ser-Ser-Ser-Thr-Pro-Leu-Leu-Tyr) was synthesized by an automated solid-phase method as described previously (Lal et al., 1991a). A cysteine residue was added to the N terminus of Env-5 in order to facilitate conjugation to proteins (see below). A series of peptides with deletions at the N and C termini of Env-5 were synthesized to identify residues critical for antibody recognition.

Antibodies to Env-5 were assayed by ELISA using isotype-specific antibodies as described previously (Lal et al., 1991a) with slight modification. Briefly, peptides were coated at 5 µg/well in carbonate buffer overnight at 4 °C. The plates were sequentially incubated with test sera at serial twofold dilution, alkaline phosphatase-conjugated second antibody (goat anti-human IgG or IgM) and p-nitrophenyl phosphate, with extensive washes between steps. Absorbance (A) was measured on an automated scanner at 405 nm. A positive result was defined as mean A of 0.1 plus 3 standard deviations for a panel of negative control sera. All sera were tested at least three times.

The Env-5 peptide was conjugated through the -SH group on the N-terminal cysteine to amino groups on keyhole limpet haemocyanin (KLH) by means of the heterobifunctional crosslinker sulphosuccinimidyl-4-(N-
maleimimidemethyl) cyclohexane-1-carboxylate (Pierce Chemicals) according to the manufacturer’s instruction. Purified HTLV antigen and Env-5-KLH at 50 µg/ml in PBS were injected intramuscularly into both hind legs of rabbits at monthly intervals. Blood was collected 3 days after the third immunization. Antibodies to Env-5 and HTLV-I were affinity-purified on Env-5-KLH-Sepharose and HTLV-I-Sepharose columns, and were coupled to fluorescein isothiocyanate by standard procedures (Hudson & Hay, 1980).

The HTLV-I syncytium inhibition assay was performed with the HTLV-I-positive C91PL cell line and the non-HTLV-I-expressing C8166 cell line which were both kindly provided by Dr Robert Gallo, NCI, NIH, Bethesda, Md., U.S.A. Cells were grown in RPMI 1640 medium with 20% heat-inactivated foetal calf serum, 5 mM-L-glutamine, 10 µg/ml gentamicin sulphate in a humidified chamber at 37°C in 5% CO₂. For the syncytium inhibition assay, 45 µl each of C91PL and C8166 cells at 1 × 10⁶ cells/ml in cell culture medium were added in duplicate to Costar 96-well microtitre plates containing 10 µl aliquots of heat-inactivated test or control serum and incubated for 24 h at 37°C. Syncytia in five fields were then counted. Control wells containing C91PL or C8166 cells only were also evaluated for syncytia. Preimmune serum samples were used as negative serum controls.

The immunofluorescence assay with acetone-fixed cells was performed using adherent slides (MM Developments) and staining with affinity-purified anti-Env-5 or anti-HTLV-I antibodies; analysis was by standard fluorescence microscopy. For fluorescence-activated cell sorter (FACS) analysis, 10⁶ cells were stained with anti-Env-5 or anti-HTLV-I for 30 min at 4°C and analysed on a FACScan (Becton Dickinson).

Immunoreactivity of anti-Env-5 antibodies was tested by immunoblotting against the HuT-78, MT-2 and Mo-T cells lines. Briefly, viral proteins (20 to 30 µg per lane) were separated on 12% polyacrylamide gels and transferred to nitrocellulose paper in 25 mM-Tris, 150 mM-glycine, 20% methanol buffer (pH 8.5). Viral proteins were detected by sequential treatment with rabbit antisera to Env-5 or with a HTLV-I-infected serum pool and 0.5 µCi of [125I]-labelled Protein G (Amersham) per sample.

For studies involving immunoprecipitation of surface proteins, labelling of MT-2 and Mo-T cells was performed using Iodo-beads (Pierce Chemicals) according to manufacturer’s instruction. The labelled cells were washed extensively in cold PBS prior to extraction in lysis buffer (0.01 M-Tris–HCl, 0.01 M-NaCl, pH 7.4, 1% NP40, 1 mM-EDTA and 0.1 mM-PMSF). Nuclei were removed by centrifugation. For immunoprecipitation, the salt concentration was adjusted to 0.15 M-NaCl and the lysate was precleared with 100 µl of a 10% suspension of formalin-fixed, heat-killed *Staphylococcus aureus* (Staph A; Pansorbin; Calbiochem-Behring). The Staph A was removed by centrifugation, and 20 µl of anti-Env-5 or anti-HTLV-I was added. Immune complexes were precipitated with 100 µl of Staph A, washed twice with lysis buffer and analysed by 12% PAGE.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Peptide sequence</th>
<th>IgG anti-peptide response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Env-5</td>
<td>C-SPNVSVPSSSTPLLY</td>
<td>18/18 (100%)</td>
</tr>
<tr>
<td>Env-5-C</td>
<td>SPNVSVPSSSTPLLY</td>
<td>18/18 (100%)</td>
</tr>
<tr>
<td>Env-5-I</td>
<td>PNVSVPSSSTPLLY</td>
<td>18/18 (100%)</td>
</tr>
<tr>
<td>Env-5-16</td>
<td>SPNVSVPSSSTPLLY</td>
<td>1/18 (5.5%)</td>
</tr>
<tr>
<td>Env-5-15</td>
<td>SPNVSVPSSSTPLY</td>
<td>0/18 (0%)</td>
</tr>
</tbody>
</table>

We have shown recently (Lal et al., 1991a) that a synthetic peptide derived from the envelope protein of HTLV-I (Env-5) reacts with 100% of serum specimens from individuals infected with HTLV-I. To characterize this response further, we have analysed IgG, IgM and IgA antibodies to Env-5 in individuals infected with HTLV-I and HTLV-II. It was found that IgG was the predominant isotype in antibody responsiveness to Env-5 with low titres of IgM and no IgA response in persons infected with HTLV-I (data not shown). As expected, none of the HTLV-II-infected or normal controls contained antibodies that reacted with Env-5.

A series of small peptides, with deletions at the N and C termini of the Env-5 peptide were used in ELISA to identify amino acids required for Env-5 reactivity. Deletion of N-terminal cysteine and serine did not affect the binding of human antibodies to the peptide (Table 1). Deletion of the C-terminal tyrosine reduced the reactivity of patient’s sera to 5-5%, whereas deletion of leucine and tyrosine residues completely abolished the binding of human antibodies to Env-5, indicating that the C-terminal residues are critical in forming the epitope detected by antibodies to Env-5.

The specificity of affinity-purified antibodies to Env-5 and whole HTLV-I were tested in ELISA. Env-5 antibodies reacted only withEnv-5 antigen in ELISA (Table 2), whereas HTLV-I antibodies reacted with Env-5, HTLV-I and HTLV-II antigens. Similarly, immunofluorescence analysis of anti-Env-5 staining of acetone-fixed MT-2 (HTLV-I-infected) or HuT-78 (uninfected) cells demonstrated specific polar fluorescence staining with the MT-2 cell line only; no specific staining of Mo-T (HTLV-II-infected) was observed (data not shown). To determine whether rabbit anti-Env-5 reacts with the native gp46 molecule, immunoblot analyses were performed on extracts of the MT-2 and Mo-T cell lines. A
serum pool from HTLV-I-infected individuals detected both \textit{gag} and \textit{env} gene products in both MT-2 and Mo-T (Fig. 1a, lanes 1 and 3), whereas rabbit anti-Env-5 specifically reacted with \textit{gp68}^\text{env} and \textit{gp46}^\text{env}, in addition to two other bands around 50K and 60K in the MT-2 cell line (Fig. 1a, lane 2), with no reactivity against the Mo-T cell line. Binding of the Env-5 antibodies to both the envelope precursor (\textit{gp68}) and the external glycoprotein (\textit{gp46}) suggests that the Env-5 peptide can mimic the antigenic epitope present on the HTLV virion. Lack of anti-Env-5 antisera reactivity with HTLV-II antigen in ELISA could be due to the orientation or the direction of attachment of peptide to the plastic solid phase.

Previous studies have shown that peptides containing prolines which can form a $\beta$ turn consistently elicit antibodies reactive with native proteins (Lerner \textit{et al.,} 1981). In accordance with those studies, we noticed that Env-5 also contained three prolines, and secondary structure analysis (Chou \& Fasman, 1978) indicated that the region corresponding to the Env-5 peptide in the native protein has a probability to form a $\beta$-turn. Absence of a similar structural motif in HTLV-II and the difference in the primary sequence of HTLV-II in the Env-5 region (Lal \textit{et al.,} 1991a) may explain the type-specific responsiveness to Env-5.

Numerous studies have indicated the potential of synthetic peptides as vaccines against viral, bacterial and protozoal infection (Lerner \& Chanock, 1984). As rabbit Env-5 antibodies reacted with the native protein, we assessed whether such antisera contained neutralizing antibodies. The rabbit immune sera did not inhibit syncytium formation in the HTLV-I-positive C91PL cell line, whereas a high titred HTLV-positive control serum completely blocked syncytium formation (data not shown). Failure to neutralize HTLV syncytium formation by antibodies to Env-5 \textit{in vitro} suggests that the dominant anti-Env-5 immune response may not be capable of inhibiting HTLV-I infectivity.

To determine whether the antigenic determinant identified by Env-5 antibodies was present on the surface of infected cells, FACS analysis performed on unfixed MT-2 cells demonstrated specific staining with anti-Env-5, whereas Mo-T cells did not stain with anti-Env-5 (Table 2). Additionally, $^{125}$I-labelled MT-2 and Mo-T cells were immunoprecipitated with either the serum pool from infected individuals or rabbit anti-Env-5. Pooled human sera precipitated a 68K molecule from both MT-2 and Mo-T cell lines; neither \textit{env} gene products (\textit{gp46} or \textit{gp21}) nor \textit{gag} gene products (p53, p24 or p19) were immunoprecipitated. Similarly, rabbit anti-Env-5 precipitated a 68K molecule (Fig. 1b, lane 2) from MT-2 and not from Mo-T, further emphasizing surface exposure of the Env-5 epitope and its specificity for HTLV-I-infected cells.

Antibodies to synthetic peptides derived from HTLV-I \textit{gp46}^\text{env} and p19\textit{ag} have been shown to react
with native viral proteins (Palker et al., 1986, 1989). One region of HTLV-1 gp46 (residues 186 to 209) contains a human cytotoxic T cell epitope (Jacobson et al., 1991), a murine helper T cell epitope (Kurata et al., 1989) and a neutralizing epitope (Ralston et al., 1989). Our study further demonstrated that the central region of gp46env defined by the Env-5 peptide also contains a surface-exposed epitope recognized by the natural host. In addition, Env-5 has recently been shown to have structural homology with growth factor receptor and induced proliferation (Lal, 1991). However, it remains to be determined whether Env-5 includes or overlaps an epitope involved in protective immunity to HTLV infection.

References


(Received 15 March 1991: Accepted 8 May 1991)