Conformational constraints of conserved neutralizing epitopes from a major antigenic area of human respiratory syncytial virus fusion glycoprotein

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To study the conformational requirements of epitopes from a conserved antigenic area (area II) of respiratory syncytial (RS) virus fusion (F) glycoprotein, peptides of increasing length containing amino acids essential for these epitopes were synthesized. The synthetic peptides were tested for binding to a panel of neutralizing monoclonal antibodies (MAbs) for this area as well as to rabbit hyperimmune and human convalescent antisera. Antibody binding was dependent on peptide length; thus, a 61-residue peptide spanning amino acids 215 to 275 of the F1 subunit (peptide F215-275) reacted with more antibodies than a shorter (41-residue) peptide F235-275, and this one with more than the (21-residue) peptide F255-275. Most human convalescent sera contained antibodies that reacted with peptides F215-275 and F235-275 but failed to react with F255-275. The results of antibody binding could be related to the structure adopted by the peptides in solution, as determined by circular dichroism spectroscopy and susceptibility of peptides to trypsin digestion. Pretreatment of peptide F215-275 with SDS abolished reactivity with certain MAbs, supporting the notion that higher order structures were needed for antibody binding. High titre anti-peptide antisera were induced in rabbits inoculated with the peptides; however, these sera failed to react with the native F molecule. In mice, only the largest F215-275 peptide induced an anti-peptide response, but their sera reacted poorly with the native F protein and the animals were not protected against an RS virus challenge. These results illustrate the potential use of synthetic peptides in studies of the F protein physical and antigenic structures as well as the problems in designing synthetic RS virus vaccines.

Introduction

Human respiratory syncytial (RS) virus is the major cause of lower respiratory tract infections in infants and young children (McIntosh & Chanock, 1990). Efforts have been made to produce inactivated and attenuated RS virus vaccines. Early trials with a formalin-inactivated virus vaccine failed to confer protection against RS virus infections. Instead, vaccinated children exhibited an exacerbated disease after a subsequent natural infection, despite the induction of neutralizing antibodies by the vaccine (Kapikian et al., 1969; Kim et al., 1969). Exacerbation of the disease was probably related to the cellular immune response against the vaccine. Thus, exacerbated disease in mice vaccinated with formalin-inactivated virus can be prevented by treatment of the mice with antibody to CD4+ T cells (Connors et al., 1992). Other attempts with live attenuated RS virus failed to induce high levels of antibodies and protection in immunized children (Belshe et al., 1982; reviewed in Norrby et al., 1989).

The reasons for the failure of formalin-inactivated RS virus to confer protection are still unknown. However, an imbalanced high ratio of ELISA-binding/neutralizing antibodies was observed in the sera of immunized children, suggesting that epitopes of the fusion (F) and/or attachment (G) viral glycoproteins were altered after formalin treatment (Murphy et al., 1986; Prince et al., 1986). These results highlight the need for a detailed characterization of RS virus antigens in order to understand the role of their epitopes in the immune response against infection and to evaluate alternative safer vaccines.

Experimental evidence suggests that the RS virus F glycoprotein is the major target of a cross-protective immune response. Thus, monoclonal antibodies (MAbs) (Taylor et al., 1984; Walsh et al., 1984) and polyclonal antisera raised against the F protein (Walsh et al., 1987),
or sera from animals inoculated with vaccinia virus recombinants expressing this antigen (Olmsted et al., 1986; Stott et al., 1987; Portela et al., 1989) neutralized RS virus. In addition, passive administration of anti-F antibodies to experimental animals (Taylor et al., 1984; Walsh et al., 1984) or immunization with either purified F protein (Walsh et al., 1987; Routledge et al., 1988) or vaccinia virus recombinants (Olmsted et al., 1986; Wertz et al., 1987; Portela et al., 1989) confer protection against a challenge with human RS virus of the two antigenic subgroups (A and B). Furthermore, antibodies to the RS virus F protein have the highest neutralization index (Anderson et al., 1988; Beeler & Van Wyke Coelingh, 1989; García-Barreno et al., 1989).

The F glycoprotein mediates both fusion of viral and cellular membranes allowing virus penetration into host cells and fusion of the membrane of infected cells with those of adjacent cells, allowing virus spread. The F protein is synthesized as an inactive precursor, F0, which is post-translationally glycosylated and cleaved into two subunits, F1 and F2, that remain linked by disulphide bonds (Gruber & Levine, 1983; Collins & Mottet, 1991). Several laboratories, using virus binding competitive assays with MAbs, have identified four or five antigenic areas on the F protein and have shown that at least three of these sites are involved in neutralization, two of which are also fusion-inhibiting (Taylor et al., 1984; Walsh et al., 1986; Trudel et al., 1987; Beeler & Van Wyke Coelingh, 1989; García-Barreno et al., 1989; Taylor et al., 1992). Recently, we have located two neutralizing antigenic areas (II and IV) on the F protein primary structure by two complementary approaches: (i) analysis of escape mutants selected with anti-F MAbs (López et al., 1990; Arbiza et al., 1992; Taylor et al., 1992) and (ii) binding of MAbs to synthetic peptides (Arbiza et al., 1992). The amino acid changes that modified the epitopes of antigenic area II included the residues 262, 268 and 272; changes at amino acid 429 altered the reactivity of MAbs recognizing antigenic area IV (Arbiza et al., 1992).

Most antibodies to antigenic area II reacted with the F1 subunit and proteolytic fragments from it in Western blots, suggesting that they recognized sequential epitopes. However, other results indicated that these 'continuous' epitopes needed a certain conformation. Thus, amino acid changes at distant locations in certain epitopes affected the binding of their MAbs and only a few epitopes could be reproduced with short synthetic peptides (Arbiza et al., 1992). To study the conformational constraints of antigenic area II, we have evaluated the binding of antibodies from this area to a set of overlapping peptides of increasing length, in an attempt to relate antibody binding with peptide conformation.

### Methods

**Virus.** The Long strain of human RS virus was used as the prototype for most experiments. It was grown in HEp-2 cells and purified from culture supernatant as previously described (García-Barreno et al., 1989).
The A2 strain was used to challenge immunized mice since it is better adapted than the Long strain to replicate in their lungs.

**Peptide synthesis.** Automated solid-phase syntheses were performed in an Applied Biosystems 430A synthesizer on a 0.5 mmol scale using p-MBHA resin as the solid support and t-Boc chemistry (Merrifield, 1986). Aspartic and glutamic acids were incorporated as Asp(OcHex) and Glu(OcHex), respectively, to minimize asparagine formation (Tam et al., 1988). Final deprotection and cleavage of peptide from the resin was achieved by the 'low-high' HF procedure (Tam et al., 1983).

Crude peptides were purified by gel filtration on Sephadex G-10 in 10% acetic acid, then loaded onto a medium-pressure Cs18 or C18 silica column equilibrated with 5% acetonitrile containing 0.05% trifluoroacetic acid (TFA) in water and eluted with a linear 5 to 60% acetonitrile gradient containing 0.05% TFA in water (Carreño et al., 1992). The fractions were analysed by reverse phase HPLC; fractions of high purity (> 90%) were pooled and lyophilized. The amino acid composition of peptides was determined after acid hydrolysis (6 M-HCl for 24 h at 110 °C) using a Beckman 6300 Analyser. Sequencing of peptides was done by automatic Edman degradation in an Applied Biosystems 477A sequenator.

**Coupling of synthetic peptides to carrier protein.** Synthetic peptides (3 mg) were coupled to 5 mg of keyhole limpet haemocyanin (KLH), previously activated with glutaraldehyde, for 16 to 18h at room temperature. The peptide-KLH complexes were purified by size exclusion chromatography on Sephadex G-50 (García-Barreno et al., 1992).

**Monoclonal antibodies and antisera.** The MAbs used throughout this study have been described previously (Taylor et al., 1984, 1992; Samson et al., 1986; Trudel et al., 1987; Kennedy et al., 1988; García-Barreno et al., 1989). Antibodies AK13A2 (Dr P. Coppe, Centre D‘Economie Rurale, Marloie, Belgium), IE3 (Samson et al., 1986) and 7C2 (Trudel et al., 1987) were kindly supplied by the respective laboratories. All MAbs were of murine origin, except MAbs B4, B5 and B10, which were produced by heterohybridomas of bovine splenocytes and murine myeloma cells (Kennedy et al., 1988; Taylor et al., 1992).

Rabbit hyperimmune antisera were raised against the peptides or against immunoaffinity-purified Long F protein (anti-F serum) after subcutaneous inoculation of New Zealand white rabbits in multiple dorsal sites (> 12) with 2 mg of either free or KLH-bound peptide or with 0.2 mg of F protein emulsified in complete Freund’s adjuvant. Three further doses of immunogen were inoculated intramuscularly in incomplete adjuvant at intervals of 10 days. Blood was collected 1 week after the last injection and processed as described previously (García-Barreno et al., 1992).

Specific pathogen-free BALB/c mice (Charles River) were immunized with 200 µg of peptide in incomplete Freund’s adjuvant intraperitoneally and boosted subcutaneously with peptide in adjuvant on a further three occasions at 3-weekly intervals. Blood was collected 3 weeks after each injection. Three weeks after the final immunizations, mice were inoculated intranasally (i.n.) with 10^5 p.f.u. of the A2 strain of RS virus as described previously (Taylor et al., 1984). Five days after challenge, the mice were killed and the lungs were homogenized and titrated for virus on secondary calf kidney cells.

The human sera obtained from children recovering from RS virus infection had been tested previously for antigen-binding competition with anti-F and anti-G MAbs (Palomo et al., 1991).

**ELISAs.** Extracts from HEP-2 cells infected with the Long strain of RS virus were prepared as described previously (Palomo et al., 1991). The RS virus cell lysate and the different peptides were tested for reactivity with anti-F MAbs or polyclonal antisera in an ELISA (Arbiza et al., 1992). Briefly, polyvinylchloride microtitre plates were coated overnight with RS virus or 2 µg of peptide at 37 °C and blocked with 5% pig serum in PBS containing 0.05% Tween-20 for 30 to 60 min. Serial dilutions of antibodies in the aforementioned blocking solution were added to the wells and the plates were incubated for 1 h at room temperature. The bound antibodies were developed using the biotin–streptavidin system and o-phenylenediamine H_2O_2 as recommended by the manufacturer (Amersham) or horseradish peroxidase-conjugated anti-mouse Ig, as described previously (Taylor et al., 1992).

In the case of bovine MAbs, bound MAbs were developed with horseradish peroxidase-conjugated anti-bovine Ig antisera. In some cases, the antigens were pretreated with increasing amounts of SDS, boiled for 5 min and diluted 10-fold with water before coating of the plates.

**Circular dichroism measurements.** Circular dichroism (CD) spectra of peptides, dissolved in 0.02 m-sodium phosphate pH 7.5, were recorded at 5 °C in 0.1 cm path-length cells in a Jasco J720 spectropolarimeter flushed with nitrogen. Each spectrum is the result of the average of three scans taken from the same sample minus the average of three scans taken from a blank reference sample. The spectra were measured between 190 nm and 260 nm using a spectral band-width of 1 nm and a scan speed of 20 nm/min.

**Tryptsin digestion.** The peptides, at 120 µM in 0.01 m-sodium phosphate pH 8.0, were incubated at 30 °C for 4 h with increasing doses of TPCk-treated trypsin attached to beaded agarose (Sigma). The digestion products were analysed by reverse phase HPLC on a C18 column eluted with a linear 5 to 70% gradient of acetonitrile containing 0.05% trifluoroacetic acid from 0.036% TFA to 0.045% TFA in water. The material in the main peaks was isolated and characterized by sequencing in an Applied Biosystems 477A sequenator.

**Results**

**Design of synthetic peptides**

We have previously shown that changes in escape mutants selected with anti-F MAbs of antigenic area II were most frequently found at residues 262, 268 and 272 of the F subunit (Arbiza et al., 1992). The proximity of these residues in the F protein primary structure, and the reactivity in Western blots of the antibodies with the F subunit and proteolytic fragments derived from it, suggested at first that these antibodies recognized linear sequences of contiguous amino acids (López et al., 1990; Arbiza et al., 1992). However, most epitopes were not reproduced with short synthetic peptides. In addition, the antigenic properties of certain escape mutants suggested that some epitopes had a conformational dependence. For instance, MAb B4 reacted with a 21-residue peptide that included amino acids 255-275 of the F subunit (peptide F255-275; see Fig. 1a and Arbiza et al., 1992). The proximity of these residues in the F protein primary structure, and the reactivity in Western blots of the antibodies with the F subunit and proteolytic fragments derived from it, suggested at first that these antibodies recognized linear sequences of contiguous amino acids (López et al., 1990; Arbiza et al., 1992). However, most epitopes were not reproduced with short synthetic peptides. In addition, the antigenic properties of certain escape mutants suggested that some epitopes had a conformational dependence. For instance, MAb B4 reacted with a 21-residue peptide that included amino acids 255-275 of the F subunit (peptide F255-275; see Fig. 1a and Arbiza et al., 1992). The location of the B4 epitope in that region correlated with the finding that mutants resistant to MAb B4 had replaced Lys272 by Thr (Fig. 1b). This amino acid change led to the loss of most epitopes from the antigenic area II of the F glycoprotein. On the other hand, escape mutants selected with MAb AK13A2 and having a single amino acid change at residue 262 (Asn to Tyr) retained reactivity with MAb B4 (Fig. 1b). However, a double escape mutant selected by MAb AK13A2 (viruses 4/4, Fig. 1b) with the change Asn262 to...
Fig. 2. Reactivity of monoclonal antibodies with synthetic peptides. (a) The binding of representative MAbs to peptides and RS virus antigen was measured in a direct ELISA, as described in Methods. Symbols are as follows: peptides F255-275 (○), F235-275 (□), F215-275 (△), F215-234 (●), F215-254 (○) and extract of human RS virus Long strain-infected cells (●). The background reactivity of each MAb with uninfected cell extract has been subtracted. The values obtained with peptide F235-254 were very similar to those with peptides F215-234 and F215-254; hence the former have not been represented. (b) Non-saturating amounts of monoclonal antibodies
Tyr and an additional change at position 216 (Asn to Asp) had lost the ability to bind MAb B4. Therefore, the B4 epitope, previously thought to be ‘continuous’ from the reactivity of MAb B4 with the ‘denatured’ F₁ subunit and peptide F255-275, was affected by a mutation in the F protein located 57 residues away from amino acid Lys272, essential for the epitope integrity.

To explore the conformational requirements of this antigenic area, peptide F255-275 was extended by 20 and 40 residues towards the N terminus to give peptides F235-275 and F215-275 respectively; the last peptide included residue Asn216. To complete the study, peptides F215-234, F215-254 and F235-254 (Fig. 1a) were also synthesized. Thus, the amino acid sequence between residues 215 and 275 was represented in several peptides of different lengths.

**Binding of antibodies to synthetic peptides**

The peptides of Fig. 1, and RS virus antigen, were used to coat the wells of microtitre plates and their binding to a panel of MAbs recognizing antigenic area II was evaluated by ELISA. The results for representative antibodies are shown in Fig. 2(a) and a summary of the antibody reactivities is presented in Fig. 2(c). None of the MAbs recognized the peptides F215-234, F215-254 or F235-254, which lacked residues 255 to 275. Binding of these peptides to the plates was confirmed by ELISA using rabbit antiserum raised against peptides F215-275 or F235-275 (see below). In addition, peptides F215-234, F215-254 and F235-254 spotted onto nitrocellulose paper could be stained with amido black, but failed to react with any of the peptides in the direct ELISA (Fig. 2a), was not inhibited by peptides (Fig. 2b). Antigen-binding of antibodies such as 47F and IE3 was inhibited more efficiently by the peptides that were recognized to higher titres in the direct ELISA. Finally, antigen-binding of antibody B4, which reacted with peptides F215-275, 235-275 and 255-275 in the direct ELISA, was also inhibited by these peptides; the shortest peptide F255-275 was the least efficient at inhibiting the antigen-binding of MAb B4.

To test whether the increased binding of MAbs to larger peptides could be reproduced with antibodies from polyclonal antisera, rabbit hyperimmune anti-F and nine human convalescent sera were tested in the direct ELISA. Representative results are shown in Fig. 3. Again, none of these sera reacted with the peptides F215-234, F215-254 or F235-254 (not shown). The rabbit antiserum raised against purified F protein (anti-F) showed a higher titre against peptides F215-275 and F235-275 than against the 21-residue peptide F255-275. The reactivity of human antisera with the peptides was variable. One antiserum (no. 215) did not react with any of the peptides. Three antisera behaved like no. 503, that is they reacted poorly with the three peptides. Five other antisera behaved like no. 512 and showed titres that were moderate against peptides F215-275 and F235-275 but very low (or absent) against F255-275. In most of these cases, the titre was higher with F215-275 than with F235-275. The reactivity of human sera with the peptides was related to their titres in a virus-binding inhibition assay done with labelled 47F antibody, as reported in Palomo et al. (1991).

The above results indicated that most monoclonal and polyclonal antibodies bound only to 41- and 61-residue peptides that spanned amino acids 255 to 275. Some of

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were incubated for 15 min at room temperature with the indicated amounts of peptides before being tested in the ELISA, using RS virus antigen-coated microtitre wells. Symbols are the same as in (a). (c) Summary of the binding results for MAbs to synthetic peptides and RS virus antigen (Long): +, > 10⁴; ±, 10² to 10⁴; --, < 10² antibody titre. ND, Not determined.
Structural studies of synthetic peptides

We have shown that the amino-terminal third of the F₁ subunit, which includes antigenic area II, is highly resistant to trypsin digestion, suggestive of a particular conformation (Arbiza et al., 1992; López et al., 1990). Thus, susceptibility of the peptides described above to trypsin digestion was evaluated as an indication of their capacity to reproduce the conformation adopted by this part of the molecule in the native F protein. After incubation with increasing doses of trypsin attached to beaded agarose, the extent of peptide digestion was estimated by HPLC analysis of their products (Fig. 4). Despite the higher content of trypsin cleavage sites in F215-275 (Fig. 1), this peptide showed a higher resistance to trypsin degradation than did peptides F235-275 and F255-275. For instance, the initial amount of peptides F255-275 and F235-275 was reduced to almost half after incubation with 6.4 milliunits (mU) of trypsin, whereas peptide F215-275 remained largely undegraded. Similarly, the final major products of peptides F255-275 and F235-275 reached their maximum values with 32 mU of trypsin whereas the maximum value of the major product from peptide F215-275 was reached with 160 mU. It is worth mentioning that the final products of peptides F235-275 and F215-275 differed in their N termini (Arg and Glu, respectively). The trypsin resistance of the Arg235–Glu236 peptide bond in peptide F235-275 is probably a consequence of its N-terminal location. The higher resistance of peptide F215-275 to trypsin degradation, compared with peptides F235-275 and F255-275, was observed in several experiments and with incubation periods ranging from 30 min to 4 h (the data of Fig. 4 correspond to incubation for 4 h).

The susceptibility of peptides to trypsin digestion was indicative of different conformations adopted by the peptides in solution. To address this point in a more direct way, CD spectra of the peptides in aqueous solution were obtained (Fig. 5). The spectra of peptides F235-275 and F255-275 were very similar. Both were suggestive of a high content of aperiodic structures, as indicated by the minimum ellipticity values at 200 nm. In contrast, the CD spectrum of peptide F215-275 showed a higher content of periodic structures (α-helix and/or β-strand) as indicated by similar minimum values of ellipticity at 205 and 222 nm and their positive value at 190 nm.

The CD results suggested that peptide F215-275 could adopt an ordered conformation in solution that can be related to its increased resistance to trypsin digestion. The conformational dependence of antibody binding was then tested in the following way. Peptide F215-275 was treated with increasing amounts of SDS and boiled for 5 min. After dilution, the reactivity of the peptide with MAbs was quantified in an ELISA. The results (Fig. 6) indicated that binding of antibodies 7C2 and 47F to the peptide was reduced by pretreatment with high doses of SDS. In contrast, the binding of other monoclonal (B4, AK13A2 or IE3) and polyclonal anti-F antibodies was essentially unaffected.

Immunogenicity of synthetic peptides

The increased antigenicity of larger peptides prompted us to evaluate their immunogenic potential. New Zealand white rabbits were inoculated with the individual peptides F215-275, F235-275 and F255-275, either free or coupled to KLH. Their sera were tested in ELISAs for reactivity with both the peptides and RS virus antigen. The results in Table 1 demonstrate that all rabbits developed high anti-peptide titres of the correct specificity. Thus, the antisera raised against peptides F215-275 and F235-275 reacted with peptide F215-254, which shared a partial sequence with the former; however, the
Conformational constraints of RSV F epitopes

Fig. 4. HPLC analysis of limited trypsin proteolytic products of the peptides. The peptides were treated with trypsin attached to beaded agarose as described in Methods. The doses of trypsin used are shown at the right. Panels I, II and III correspond to the analysis of peptides F255-275, F235-275 and F215-275 respectively. The initial peptide peaks are denoted by triangles (•) and the final products by asterisks (*). The final products of panels I, II and III were purified by HPLC and sequenced. The sequences for their amino and carboxy termini are as follows: 255 SELL...DQKK 272 (I); 235 REFS...DQKK 272 (II); 236 EFSV...DQKK 272 (III).

latter peptide was not recognized by anti-F255-275 antiserum. Despite the high immunogenic potential of the peptides to induce anti-peptide antibodies in rabbits, none of these antisera reacted with the RS virus F protein (Table 1, Long).

Mice immunized with peptide F215-275 developed high titres of antibody to peptides F215-275, F235-275, F255-275, F215-234 and F215-254, 3 weeks after the last immunization (Table 1). In contrast, none of the mice immunized with peptides F235-275 or F255-275 developed anti-peptide antibodies, even after four doses of peptide. Despite the high level of anti-peptide antibodies induced by peptide F215-275, the mouse sera reacted poorly with RS virus (the RS virus A2 strain was used for the experiment shown in Table 1, but similar results have been obtained with the 8/60 strain of human RS virus and two bovine strains). Furthermore, there was no evidence of protection against infection in mice immunized with any of the peptides, 5 days after i.n. challenge with RS virus (data not shown). It is worth mentioning that the anti-peptide titre was observed after the first inoculation of F215-275, whereas the antibodies against RS virus were detected only after the fourth inoculation of peptide.

Discussion

The main goal of this study was to investigate the structural requirements of antigenic area II, which is apparently an immunodominant region of the RS virus F glycoprotein recognized by different animal species. A large number of murine MAbs that recognize epitopes of this area have been isolated independently (Taylor et al., 1984, 1992; Trudel et al., 1987; Garcia-Barreno et al., 1989). Epitopes recognized by bovine MAbs (Kennedy et al., 1988) have also been mapped in antigenic area II (Taylor et al., 1992). Finally, human post-infection sera and rabbit hyperimmune antiserum contain antibodies that compete with MAbs for binding to antigenic area II (Palomo et al., 1991) and that react with synthetic peptides from this area (Fig. 3). Previous failures to identify reactivity of human antisera with synthetic peptides in antigenic area II could be explained by the use of short peptides in those studies (Scopes et al., 1990), as can be inferred from the results of Fig. 3.

The results of antibody binding to synthetic peptides illustrate the structural complexity of antigenic area II (Fig. 2). Some antibodies, such as 11, 13, 49F and B10, did not react with any of the peptides indicating that
either the antibody-binding sites were not represented in the peptide sequences or that they required the native protein conformation for binding. We favour the second possibility for two reasons: (i) binding of those antibodies was lost in escape mutants with amino acid changes at positions 262, 268 or 272 and (ii) those antibodies did not react in Western blots with the F1 subunits (Arbiza et al., 1992; Taylor et al., 1992). One antibody (B4) reacted to high titre with the 21-residue peptide F255-275 [in fact epitope B4 also binds to the octapeptide F266-273 (P. Whyte, T. Doel & G. Taylor, 1992)].

Table 1. *Immunogenicity of peptides in rabbits and mice*

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* Long and A2 strains of RS virus were used in these experiments to test rabbit and mouse antisera, respectively; however, the values of antiserum titres obtained in other experiments were similar with either strain.
† ND, Not determined.
unpublished). Other antibodies (F3, AK13A2, IE3 and B5), however, did not bind to F255-275 but reacted with peptide F235-275, although they showed greater binding to peptide F215-275. Finally, MAbs 47F, 7C2 and 18 reacted only with the 61-residue peptide F215-275. Considering the length of this peptide, structural features other than primary sequences should be necessary for antibody recognition. In agreement with this hypothesis, the binding of antibodies 47F and 7C2 was inhibited by previous treatment of the peptide F215-275 with SDS and heating (Fig. 6). Under these conditions, however, the binding of antibodies whose epitopes were reproduced in the shortest peptide (e.g. B4) was essentially unaffected.

The increased antigenicity of larger peptides that included the amino acid sequence 255 to 275 was correlated with the conformation adopted by the peptides in solution. Thus, the 61-residue peptide F215-275, which reproduced more epitopes, was more resistant to trypsin digestion (despite having more cleavage sites) than the shorter peptides. This characteristic is reminiscent of the trypsin resistance of the F, subunit N-terminal third (residues 136 to 290) (López et al., 1990). The structure adopted by this region of the F glycoprotein must be stable under the harsh conditions of Western blotting, or able to renature after electrotransfer to the immunoblot paper, since some antibodies (e.g. 47F) that recognized the F subunit in Western blots lost reactivity with F215-275 upon SDS and heat treatment (Fig. 6). Secondary structure prediction for the F protein favours the presence of two α-helices in the region included in peptide F215-275 (Fig. 1). This could explain the higher content of ordered structures of this peptide, compared with peptides F235-275 and F255-275, as deduced from the CD spectra (Fig. 5). Determination of the three-dimensional structure of peptide F215-275, which is more amenable to structural studies than the entire F molecule, could provide information about the conformation adopted by this region of the F protein in the native molecule.

The results of antigen binding of MAbs 18, 47F and 7C2 have precedents in other viral proteins. Wagner et al. (1992) have reported recently that certain epitopes of the human cytomegalovirus gp58 glycoprotein require a continuous sequence of more than 70 amino acids. Human and murine MAbs that recognized the sequence reacted in Western blots with the gp58 protein.

We do not know the actual residues of the RS virus F glycoprotein that interact with antibodies, such as 47F. There are two alternative explanations for the peptide binding requirements of antibodies like this. First, the residues that make contact with the antibody are included in the region of residues 255 to 275, where mutations selected in viruses resistant to that antibody are located, but the entire 215 to 275 sequence is needed to adopt the appropriate conformation for antibody binding. Second, the residues that make contact with the antibody are scattered throughout the entire 215 to 275 sequence but they need to adopt a given conformation for antibody binding. However, it should be borne in mind that none of the MAbs tested reacted with peptides F215-234, F235-254 or F215-254 which lacked residues 255 to 275.

Despite the capacity of peptide F215-275 to reproduce a large number of epitopes and the high immunogenic potential of peptides F215-275, F235-275 and F255-275 in rabbits, none of their antisera reacted with the native F protein. Strikingly, only the largest peptide (F215-275) induced an anti-peptide response in mice, but their sera reacted poorly with the virus and the animals were not protected against an RS virus challenge. This finding parallels the limited success of other authors in inducing anti-F antibodies using synthetic peptides with sequences included in antigenic area II (Bourgeois et al., 1991; Trudel et al., 1991). Thus, the induction of a protective humoral immune response with peptides remains a major obstacle to the design of an RS virus synthetic vaccine. However, the different response of rabbits and mice to peptides and the low titre of anti-F antibodies detected in mice inoculated with F215-275 deserve further investigation. It is possible that larger peptides from antigenic area II (or protein fragments) that reproduced epitopes such as those recognized by MAbs 11, 13, 49F and B10, which did not react with the peptide set of this study, might induce or potentiate an antiviral response. Nevertheless, synthetic peptides have been successfully used to study the structure of certain proteins and protein domains (Hodges et al., 1988; Van der Graaf & Hemminga, 1991; Zimmermann et al., 1991). The results presented here illustrate their potential application in structural studies of the RS virus F molecule.

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