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Evidence that the amino acid region 124–203 of glycoprotein G from the respiratory syncytial virus (RSV) constitutes a major part of the polypeptide domain that is involved in the protection against RSV infection

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Abstract

The first 230 residues of the 298-amino acid glycoprotein G of respiratory syncytial virus (RSV) are sufficient to confer complete resistance to challenge with live RSV, whereas the first 180 residues completely failed (Olmsted et al. (1989) J. Virol. 63, 411–420). The characterization of a protective epitope corresponding to the amino acid region 174–187 of the G protein (Trudel et al. (1991) Virology 185, 749–757) suggests that interruption of this region in the 180 residue truncated polypeptide may be responsible for its inability to confer protection and consequently that the 174–187 region may play a major role in the protection effected by the protein G. To support these hypotheses, we examined the ability of the amino acid region 124–203 of glycoprotein G to confer protection. The corresponding peptide was expressed as a non-fusion protein in a recombinant vaccinia virus designated VG27. Immunization of BALB/c mice with this recombinant efficiently induced the production of antibodies capable of recognizing both the parental glycoprotein G and peptide 174–187. Furthermore, upon challenge with RSV, a significant decrease of infectious particles was found in the lungs of mice immunized with VG27 as compared with non-immunized mice. Our results suggest that the 124–203 amino acid region of the RSV G protein constitutes a major part of the domain involved in protection.

Keywords: Respiratory syncytial virus (RSV); Polypeptide G, truncated; Recombinant vaccine

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1. Introduction

Human respiratory syncytial virus (RSV) is a major cause of viral pneumonia and bronchiolitis in infants and young children. So far, 10 viral proteins have been described (for a review, see McIntosh and Chanock, 1990). The G glycoprotein of RS Long strain virus contains 298 amino acid residues with a calculated MW of 33 kDa (Johnson et al., 1987b), however the mature polypeptide migrates as a 88-kDa species in SDS–PAGE because of extensive O-linked and N-linked glycosylation. The polypeptide sequence contains 8 potential acceptor sites for N-linked sugars and more than 70 potential sites for O-glycosylation. It was recently shown that synthesis of glycoprotein G is initiated at either of two alternative AUGs codons from a single mRNA species for the production of two different mature forms of the protein, an anchored type II integral membrane form and a smaller form which is secreted into the medium (Roberts et al., 1994). Immunization studies, using either purified glycoprotein G or recombinant vaccinia viruses expressing the protein, have demonstrated that it represents a major independent antigen and has the ability to confer complete resistance to RSV infection (Elango et al., 1986; Stott et al., 1986; Johnson et al., 1987a; Walsh et al., 1987; Olmsted et al., 1988, 1989; Murphy et al., 1989; Trudel et al., 1989, 1992; Sullender et al., 1990; Connors et al., 1991, 1992). Furthermore, Olmsted et al. (1989) have shown, using recombinant vaccinia virus G230 expressing the first 230 amino acid residues of the polypeptide, that these were sufficient to confer complete protection to live RSV, whereas recombinant G180 expressing the first 180 residues failed completely. These findings suggest that the region situated within positions 180 and 230 is involved in protection. Other studies reported the characterization of epitopes involved in immunity and protection (Norrby et al., 1987; Akerlind-Stopner et al., 1990; Trudel et al., 1991; Garcia-Barreno et al., 1992). In particular, we have shown that BALB/c mice immunized with a synthetic peptide representing the amino acid region 174–187 were completely resistant to RSV replication (Trudel et al., 1991). However, this peptide had to be coupled to a macromolecule in order to induce immunity effectively. Interestingly, the epitope 174–187 is present in the recombinant G230 which fully protected, whereas it was interrupted in the recombinant G180 which did not protect at all. We hypothesized that the inactivity of the latter recombinant was due to the interruption of the 174–187 region and as a consequence, that this region played a major role in the protection observed with the G230 recombinant. To verify this hypothesis we investigated the protective activity of the amino acid region 124–203 of the G protein following its expression as a non-fusion protein in a recombinant vaccinia virus designated VG27. The resulting 86-mer peptide containing 80 residues of the G protein was synthesized at the expected size (10 kDa) in VG27-infected cell cultures. Mice immunized with VG27 responded with specific antibodies and their lungs were significantly resistant to replication of RSV, upon challenge. The partial protective efficacy we observed relative to other G recombinants is discussed with regard to the level of expression as well as the location of the polypeptide in infected cells. Our results suggest that amino acid residues 124–203 of protein G represent a major part of the domain involved in protection.

2. Materials and methods

2.1. Cells, virus and viral mRNA

The human Long strain (ATCC VR-26; American Type Culture Collection, Rockville, MD, USA) of RSV was propagated in HEp-2 cells (ATCC CCL-23), as previously described (Trudel et al., 1991). Viral mRNA was isolated from cells at 20 h postinfection under conditions reported elsewhere (Simard et al., 1990).

Vaccinia virus (strain WR, ATCC VR-119) was obtained from ATCC and cultured in HeLa cells (ATCC CCL-2). Recombinant vaccinia viruses were selected on TK^- 143 cells (ATCC CRL-8303) in the presence of 15 μg/ml of 5-bromodeoxyuridine (BrdU).

2.2. Coexpression vector pUV1 / ter

The coexpression vector pUV 1 was generously provided by Dr. Bernard Moss (National Institute of Allergy and Infectious Diseases, Bethesda, USA, MD 20892). The plasmid includes the *E. coli* ß-galactosidase gene regulated by the vaccinia P7.5 promoter to facilitate screening of viral recombinants. It also contains an initiation codon downstream from the vaccinia P11 promoter and upstream from 6 useful cloning sites (Falkner et al., 1987). The vector was further modified to contain termination codons in any reading frame, downstream from the ATG codon. For this purpose, *Sma*I digested pUV 1 plasmid DNA was ligated with a phosphorylated *Nhe*I linker (Pharmacia/LKB). The reaction mixture was then extracted twice with phenol and DNA digested with an excess of *Nhe*I restriction enzyme. After fractionation of DNA in agarose gels, the 8.5-kbp fragment was isolated, self-ligated and used to transform competent *E. coli* cells. First, recombinants were selected for gain of *Nhe*I and loss of *Sma*I restriction sites. One such clone, designated pUV1/ter, was obtained and sequenced by the method of Maxam and Gilbert (1977).

2.3. Construction of vaccinia recombinants

The DNA region encoding amino acid residues 124–203 of RSV glycoprotein G was amplified by PCR following reverse transcription of mRNA. Synthesis of cDNA was primed with the oligonucleotide 3'-TGGTGGTGGTTCGGATGCCTAGGCC-5' representing nucleotide positions 591–608 of the glycoprotein G coding region, followed by a *Bam*HI restriction site (underlined). Reverse transcription was performed at 42°C for 1 h in 20 μl of 20 mM Tris-HCl pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$ and 0.5 mM dNTP containing 2 μg of total RNA isolated from RSV-infected cells, 5 pmol of primer and 13 U of AMV reverse transcriptase (Pharmacia/LKB). Synthesized cDNA was then amplified by PCR using the above primer and a second oligonucleotide 5'-CGGAATTCAAACCTGCAACCCACA-3' representing nucleotide positions 370–387 of the RSV glycoprotein G coding region preceded by an *Eco*RI restriction site (underlined). Eighty μl of a solution containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.001% gelatine, 10 pmol of each primer and 2.5 U of AmpliTaq™ DNA polymerase (Perkin Elmer Cetus) was added to the cDNA reaction

mixture. The reaction tube was incubated in a Perkin Elmer Cetus thermal cycler set at 30 cycles of denaturation (94°C, 1 min), annealing (55°C, 40 s) and extension (72°C, 1 min). The mixture was then extracted once with chloroform : isoamyl alcohol (24 : 1), DNA was precipitated with ethanol, dissolved in H_2O and digested with an excess of *Eco*RI and *Bam*HI restriction enzymes. The DNA was then fractionated in 2% agarose gels, purified and ligated with pUV1/ter plasmid DNA previously digested with *Eco*RI and *Bam*HI. Upon transformation of *E. coli*, recombinant bacteria were selected for presence of a 238-bp *Bam*HI/*Eco*RI fragment. Finally, the positive clone pUV1/ter-G27 was sequenced.

Nearly confluent (80%) monolayer HEp-2 cells previously infected with wild-type vaccinia virus at an m.o.i. of 2, were transfected with cesium chloride-purified pUV1/ter or pUV1/ter-G27 plasmids using Lipofectin™ reagent (Gibco/BRL). TK^- recombinants were amplified by two passages of cellular lysates on TK^-143 cells in the presence of BrdU. Recombinants were then selected, for TK^-/ß-Gal^+ phenotypes, in plaque assays using 300 μg/ml of 5-bromo-4-chloro-3-indolyl-ß-D-galactoside in the 1.5% agar overlay. Blue plaques were picked and selected again as before. Recombinant viruses VTer and VG27 (corresponding to transfected pUV1/ter or pUV1/ter-G27 plasmids, respectively) were produced on HEp-2 cells. The presence of RSV glycoprotein G coding region in VG27 was ascertained by Southern blot hybridization of viral DNA purified as described (Simard et al., 1990, 1991). Expression of the G peptide was analyzed in Western blots of polypeptides obtained from infected cell lysates, using an immune serum directed against RSV.

2.4. Immunization and protection assays

BALB/c mice required in these experiments were cared for in accordance with approved guidelines. The protocol used was reviewed and approved by the animal care committee of the Institut Armand-Frappier. Four groups of either 3 or 6 BALB/c mice (female, 3–4 weeks old) received one intraperitoneal injection of 1.5×10^6 PFU (0.3 ml) of either VG27 or VTer recombinant vaccinia viruses or of 9.5×10^6 PFU of RS Long virus. Two weeks after immunization, mice were challenged intranasally with 1.5×10^5 PFU of RS Long virus. Five days later, the animals were anesthetized and their blood and lungs were collected. ELISA and Western blot assays using polypeptides obtained from purified RSV as antigens and neutralization assays without complement were performed as previously described (Trudel et al., 1985, 1987). Lungs were homogenized at 0°C in 10 vols. of 199 Hank's, 25 mM HEPES pH 7.8, 218 mM sucrose, 30 mM $MgCl_2$ containing 0.5 μg/ml of fungizone and 50 μg/ml of gentamycin, using a manual homogenizer. After centrifugation at 1500 rpm for 15 min, supernatants were collected and tested for the presence of virus by titration assays. These were carried out in quadruplicate in microtiter plates (24 wells) containing HEp-2 monolayer cells seeded 2 days previously at 1×10^5 cells/well. Culture medium was removed from confluent cultures and 200 μl of 10-fold viral supernatant dilutions were added. After a 2-h adsorption period at 37°C in 5% CO_2 atmosphere, 1.5 ml of medium containing 199 Hank's : Earle's MEM (1 : 1), 1% fetal bovine serum and 50 μg/ml gentamycin was added and plates were incubated for 5 days. Titer of virus was

expressed as $\log_{10}$ $TCID_{50}$/g of lung (Taylor et al., 1984). Statistical analysis using the *t*-test (Cricket Software, Inc., Philadelphia, PA 19104, USA) was performed to determine significant differences.

3. Results

We generated a pUV1/ter coexpression vector from the parental pUV 1 vector. Besides the presence of a translation initiation codon included in the latter (Fig. 1A), the derived pUV1/ter vector provided termination codons in all 3 possible reading frames (Fig. 1B). This feature ensures termination of polypeptides that could be initiated at other ATG(s) contained in exogenous DNA sequences, while facilitating the construc-

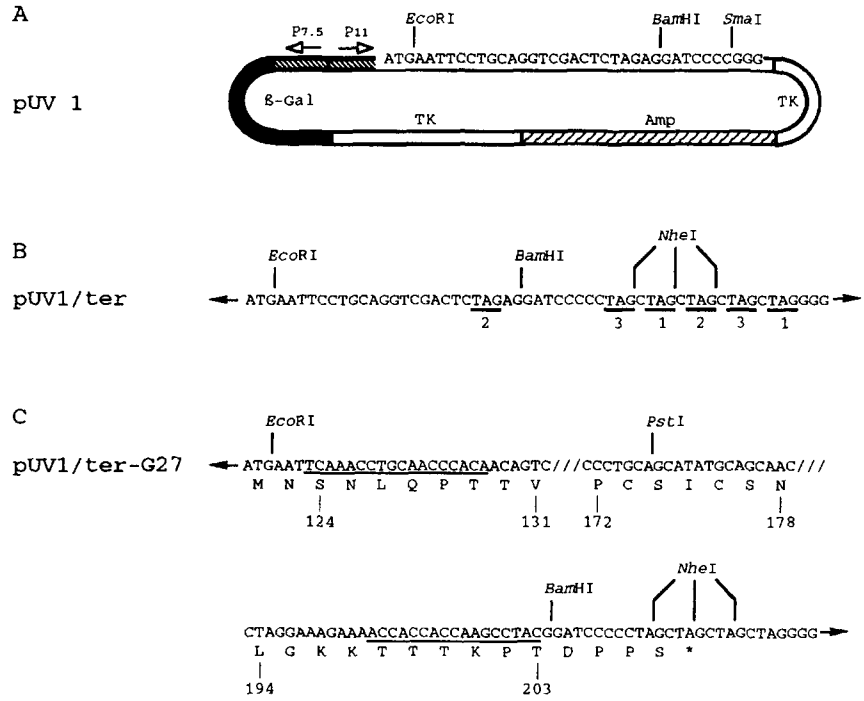

Fig. 1. Coexpression vectors and recombinant plasmids. A: diagram of pUV 1 showing the nucleotide sequence of the region corresponding to the cloning sites preceded by the translation initiation codon ATG (Falkner et al., 1987). The plasmid was used to generate the vector pUV1/ter (B), containing termination codons (underlined) in all 3 reading frames (as indicated) relative to the ATG codon. The DNA region encoding amino acid residues 124–203 of RS virus glycoprotein G was inserted in frame in pUV1/ter to generate the recombinant pUV1/ter-G27 (C). The DNA and predicted amino acid sequences of the relevant region are partially presented, slashed bars representing intervening sequences. Amino acid residues are numbered relative to the parental G glycoprotein. Regions corresponding to the primers used for amplification of the coding sequence are underlined. Inserted DNA was chemically sequenced starting at the unique *Pst*I restriction site.

tion of viral recombinants for the expression of internal regions of polypeptides of interest as non-fusion proteins. The pUV1/ter vector used in this study contained 3 *Nhe*I restriction sites in tandem generating one stop codon in the second reading frame and two in the other two, relative to the vector's ATG. An additional stop codon in the second reading frame was also located upstream from the *Bam*HI restriction site.

The DNA region 370–608 of RSV glycoprotein G coding sequences representing amino acid residues 124–203 was amplified and inserted in the *Eco*RI and *Bam*HI unique restriction sites of pUV1/ter vector. Chemical sequencing of the resultant recombinant plasmid pUV1/ter-G27 starting at the unique *Pst*I site of the amplified DNA region (nucleotide 520 relative to the glycoprotein G coding region) confirmed that the RSV coding region was intact and in frame with the vector's ATG codon (Fig. 1C). Finally, as a result of the cloning procedure, the translated truncated polypeptide also contained small border regions consisting of two and four residues at the amino and carboxy termini, respectively, that were not represented in the amino acid sequence of RSV glycoprotein G.

3.1. Construction of recombinant vaccinia virus

Mammalian cells previously infected with wild-type vaccinia virus were transfected with either pUV1/ter-G27 or pUV1/ter plasmid DNA to generate $TK^-/ß\text{-}Gal^+$

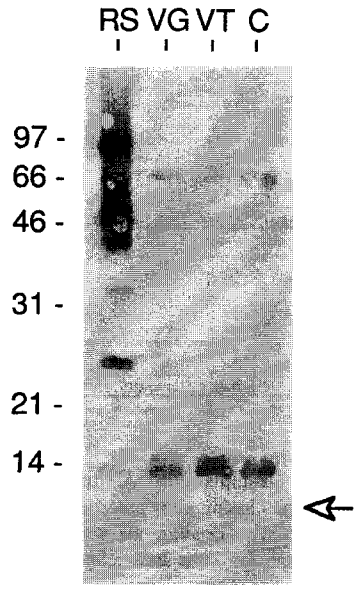

Fig. 2. Western blots prepared from cell lysates. Lysates of either mock-infected (C), VTer (VT)- or VG27 (VG)-infected cells were fractionated in 15% polyacrylamide gels. Following electrotransfer, the blots were reacted with an immune serum directed against RSV. Lane RS represents analysis of purified RSV. Molecular weights of protein standards are indicated in kDa. Arrows identify the 87-mer peptide expressed in VG27 recombinant vaccinia virus.

recombinant vaccinia viruses VG27 and VTer, respectively. Southern blot hybridization of *Bam*HI/*Eco*RI digests of purified viral DNA using pUV1/ter-G27 as a probe allowed the detection of a fragment in the size range of 240 bp in the VG27 DNA sample, thus confirming that the VG27 recombinant virus included the coding region 370–608 from the RSV glycoprotein G (results not shown).

Expression of the polypeptide region 124–203 from RSV glycoprotein G in cells infected with VG27 was analyzed by Western blot analysis of infected cell lysates (Fig. 2). Using immune serum directed against whole RSV, lysates of either non-infected (lane C), VTer (lane VT) or VG27 (lane VG) infected cells revealed the presence of two polypeptides of 66 and 13 kDa, suggesting cross-reaction with cellular polypeptides. However, by contrast to lanes C and VT, a very faint band corresponding to a polypeptide in the range of 10 kDa was detected in lane VG. Due to the weak intensity of the band, the Western blot assay was repeated to confirm these results (not shown). The 10-kDa polypeptide undoubtedly represented the 86-mer RS peptide synthesized in VG27-infected cell, since it specifically reacted with the RS immune serum and was of the expected size (9.67 kDa). The weak intensity of the band may either indicate that the RSV immune serum only contained few antibodies recognizing linear epitopes of the G 124–203 region, that the expression of the truncated polypeptide in cell culture was low, or that the polypeptide was secreted in the supernatant of VG27-infected cells.

3.2. Immunological and protection assays

The ability of recombinant VG27 to induce humoral immunity and confer protection was examined in BALB/c mice. In ELISA assays, titers obtained from sera of mice immunized with either recombinant VG27 or whole RSV were as good (Table 1). However, neither serum revealed the presence of neutralizing antibodies in neutralization plaque assays (results not shown). Upon intranasal challenge with RS Long virus, lungs of individual mice immunized with the recombinant VG27 contained a residual mean titer of 200 ($\log_{10}$ 2.3) infectious RSV particles/g compared with 4000 ($\log_{10}$ 3.54) in the lungs of either non-immunized or VTer-immunized mice (Table 1). Interestingly, two of the six mice immunized with the VG27 recombinant had no detectable RSV particles in their lungs after challenge. These results clearly indicate that immunization with the recombinant VG27 significantly reduced the ability of the RSV to replicate in mice upon challenge.

The production of anti-glycoprotein G antibodies in VG27-immunized mice was demonstrated in Western blot assays of RSV proteins (Fig. 3A) and in dot-blot assays of peptide 174–187 directly adsorbed onto nitrocellulose membranes (Fig. 3B). The patterns of polypeptides detected in Western blot assays using sera from mice immunized with either VTer (Fig. 3A, lane 3) or VG27 (lane 4) recombinant viruses were similar with the exception that the latter serum also revealed the presence of an abundant polypeptide of 90 kDa. The size and the thickness of this band corresponded to that observed for the glycoprotein G of RSV following its detection with the RSV specific antiserum (lane 1). These results unambiguously confirm that the VG27 immune serum was capable of recognizing the parental G protein. Bands that were common in lanes 3 and 4 represented cross-reaction of vaccinia virus-specific antibodies with either viral or

Table 1
Protection conferred in mice against RSV following their immunization with the VG27 recombinant virus

Assay	Titer of RS virus [a]	Mean ± S.D.	*P*-value [b]	ELISA [c]
VG27	2.8	⩽ 2.3 ± 0.5	0.001	3600
	2.5			
	2.5			
	2.5			
	⩽ 1.7			
	⩽ 1.7			
VTer	3.5	3.6 ± 0.2	1.0	800
	3.5			
	3.8			
RSV	⩽ 1.7	⩽ 1.7	⩽ 0.001	6400
	⩽ 1.7			
	⩽ 1.7			
I^-/C^+ [d]	3.5	3.6 ± 0.2		200
	3.8			
	3.5			
I^-/C^- [e]	⩽ 1.7	⩽ 1.7		200
	⩽ 1.7			

Groups of 6 or 3 mice were immunized with either VG27 or VTer recombinant vaccinia viruses or RSV. Two weeks later, animals were challenged intranasally with RS Long virus. Five days later, lungs were collected and tested for the presence of virus by titration assays.

[a] Titer of virus was expressed as $\log_{10}$ $TCID_{50}$/g of lung.

[b] The *P*-value was calculated from comparison with I^-/C^+ mice.

[c] Mean titers were expressed as the last dilution giving an optical density two-fold above background.

[d] I^-/C^+, non-immunized mice submitted to challenge.

[e] I^-/C^-, non-immunized and non-challenged mice.

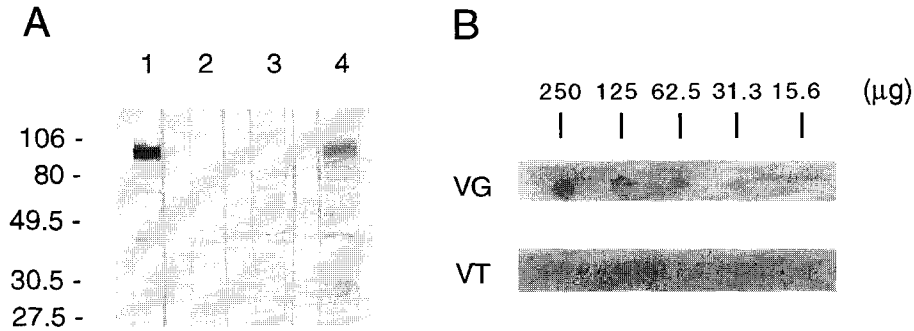

Fig. 3. Analyses of immune sera. A: Western blot detection of RSV polypeptides using sera from mice immunized with either RSV (lane 1), VTer (lane 3) or VG27 recombinant vaccinia viruses (lane 4). Lane 2 represents a negative control performed with serum from non-immunized mice challenged with RSV. Molecular weights of protein standards are indicated in kDa. B: Dot-blot detection of various quantities (μg) of peptide 174–187 adsorbed onto nitrocellulose membranes, using sera from mice immunized with either VTer (VT) or VG27 (VG) recombinant viruses.

cellular proteins. Finally, only the VG27 serum was able to specifically detect peptide 174–187 in dot-blot assays (Fig. 3B). The level of detection was dose dependent with a detection limit situated between 31.3 and 15.6 μg of peptides. Our results demonstrate that the 124–203 amino acid region of RSV glycoprotein G was efficiently expressed in mice immunized with the VG27 virus and induced the production of specific antibodies capable of recognizing the parental glycoprotein G polypeptide and its 174–187 amino acid region.

4. Discussion

Using recombinant vaccinia viruses G230 and G180, Olmsted et al. (1989) demonstrated that the first 230 residues of the glycoprotein G of RSV were sufficient to confer complete resistance to infection with live RSV, while the first 180 residues failed completely. The characterization of a linear protective epitope represented by the amino acid region 174–187 (Trudel et al., 1991) raises the two following questions: Is interruption of the protective epitope 174–187 in the recombinant G180 responsible for its inability to confer protection? If so, can we conclude that the region represented by amino acids 174–187 plays a major role in the protective efficacy of the glycoprotein G? To answer the first question, the activity of a mutant protein G terminating just downstream from position 187 could be analyzed. However, this would not exclude the possibility that the N-terminal regions and carbohydrates linked to the polypeptide are involved in protection. Consequently, we decided to analyze the approximate central region of the protein G. The region selected for this purpose represented the amino acid residues 124–203 and included the epitope 174–187. The corresponding 80-mer peptide was expressed as a non-fusion protein in a recombinant vaccinia virus (VG27) to facilitate comparisons with the two recombinants G230 and G180. The 124–203 amino acid region selected corresponded approximately to the middle portion of the predicted extracellular domain of the protein G and included a single small hydrophobic region (residues 167–177) as well as potential acceptor sites for N-linked (one site) and O-linked carbohydrates (20 sites) (Johnson et al., 1987b). In spite of the presence of these features, post-translational processing was not expected to occur due to the absence of the transmembrane anchor region (residues 38–66). The role of the latter in targetting the protein for glycosylation was confirmed from analysis of a SV40 recombinant virus expressing the amino acid residues 1–71 of RSV glycoprotein G (Olmsted et al., 1989). In the present study, synthesis in cell culture of the 86-mer peptide comprising 80 residues from the G polypeptide, generated a polypeptide in the range of 10 kDa as expected from its primary structure (9.67 kDa), indicating no evidence of post-translational modification. However, immunoprecipitation of cell lysates following metabolic labeling with [^{3}H]glucosamine is required to confirm this.

Immunization of mice with the recombinant vaccine VG27 conferred significant resistance to RSV replication in their lungs following challenge. Indeed, the recombinant allowed the reduction of the virus titer by a mean factor of 20 (1.3 $\log_{10}$) as compared with non-immunized or VTer-immunized mice. Interestingly, two of the six mice employed in the assay were completely protected. As was observed following immuniza-

tion with the synthetic peptide 174–187 (Trudel et al., 1991), the protective activity of the VG27 recombinant did not involve the production of neutralizing antibodies. By contrast to the G230 recombinant (Olmsted et al., 1989), the VG27 recombinant was unable to confer complete protection to immunized animals. The differences in the level of protection conferred by the two recombinants may be explained by differences in antigen presentation, as the 230-amino acid polypeptide was shown to accumulate at the surface of infected cells with its carboxy-terminus oriented extracellularly, while the 86-mer peptide would most likely accumulate in the cytoplasm of infected cells. Additionally, differences in the levels of expression of the two polypeptides may be involved, since the late P11 and the strong early/late P7.5 vaccinia promoters regulate the 86-mer peptide and 230-amino acid polypeptide, respectively. This postulate is consistent with preliminary studies showing that mice immunized with the 124–203 peptide synthesized in a prokaryotic expression vector developed complete resistance to RSV replication (in preparation). These results also indicate that differences in the level of glycosylation of the 10-kDa peptide versus the 51-kDa polypeptide generated by recombinants VG27 and G230, respectively, does not account for the differences in the levels of protection conferred. Despite the partial resistance conferred by the recombinant VG27, the activity of the 124–203 amino acid region of the protein G still appears to be of great interest, considering that the size of the corresponding peptide was less than 20% that of the polypeptide generated by the G230 recombinant. This indicates that eventually, a peptide of minimum size with the ability to induce immunity by itself and to confer complete protection could be entirely derived from the glycoprotein G of RSV for the development of a human vaccine (see below).

By comparison with the mutant G180 polypeptide which completely failed to confer resistance to RSV, our results show that significant protective activity can be retrieved following addition of the subsequent 23 residues at the C-terminal end of the polypeptide, and even though the first 123 residues are deleted. This suggests that the 124–203 amino acid region of the protein G of RSV represents a major part of the domain involved in protection. Moreover, the presence of the protective epitope 174–187 (Trudel et al., 1991) in both VG27 and G230 recombinants and its absence in the G180 recombinant lead to the hypothesis that this small sequence may play an important role in protection. However, other regions shared by the 124–203 peptide and the 230-amino acid polypeptide may also participate in their protective activity.

It should be mentioned that the role of the RSV major antigens in protection is controversial since the simultaneous use of F- and G-vaccinia recombinants to immunize chimpanzees and owl monkeys gave contradictory results. Indeed, the two recombinants were unable to protect immunized chimpanzees against RSV challenge (Collins et al., 1990; Crowe et al., 1993), whereas they conferred a great degree of resistance to infection in owl monkeys (Olmsted et al., 1988). This is important because among experimental animals, only the chimpanzee and owl monkey develop symptoms of RSV disease (McIntosh and Chanock 1990). Nevertheless, as hypothesized by Collins et al. (1990), the vaccine failure in chimpanzees could be specific to this species, to the use of the vaccinia virus vector or to insufficient antigen. Fortunately, it was recently reported that seropositive children developed good immune responses following immunization with an RSV F protein subunit vaccine (Tristram et al., 1993, 1994; Belshe et al., 1993;

Welliver et al., 1994). Interestingly, even though the vaccine only contained a small amount of glycoprotein G (~ 5%), strong G-specific responses were induced in the serum of vaccinees. Furthermore, we have demonstrated that immune sera from naturally infected individuals reacted specifically with a peptide representing the epitope 174–187 (unpublished results). These results indicate that the polypeptide-G, and more specifically the epitope 174–187, represent a relevant target in the natural host for the development of a potential vaccine. In spite of these encouraging results, the widespread use of a protein G-based vaccine including the epitope 174–187 may still appear questionable, due to the relatively high sequence divergence of the polypeptide in different subgroup isolates. Nonetheless, it was found that RSV mutants (subgroup A) resistant to neutralization by monoclonal antibodies directed against glycoprotein G only contained mutations downstream of amino acid 192 (Garcia-Barreno et al., 1990; Rueda et al., 1991). Furthermore, analysis of the genetic diversity of glycoprotein G of either naturally occurring subgroup B strains isolated from infected children over a 30-year period (Sullender et al., 1991) or natural subgroup A strains isolated during a single epidemic (Cane et al., 1991) detected no variant in the 174–187 amino acid region. These studies as well as earlier sequence comparisons of different isolates (Johnson et al., 1987b; Sullender et al., 1990) demonstrate that the 174–187 region is highly conserved in subgroup A as well as in subgroup B isolates. Clearly, these observations are consistent with the widespread use of a protein G-based vaccine.

The data presented herein may open the way to the development of a biosynthetic peptide vaccine entirely derived from the G glycoprotein and which would include only those polypeptide regions that are required for inducing immunity and protection. As the vaccinia virus is not expected to become a suitable vector for the development of human vaccines in the near future, we are currently pursuing evaluation of the potency of the 124–203 amino acid region as well as of slightly larger areas of the G protein following their expression in a prokaryotic vector and the results obtained to date are more than promising (in preparation). We are also evaluating the role of specific amino acid residues suspected of playing an important role in protection via the use of site-directed mutant peptides. Finally, it will be of great interest to establish whether the 124–203 amino acid region of polypeptide G confers cross-protection against subgroup B isolates and, if necessary, the protective potency of the corresponding region in subgroup B isolates will have to be determined.

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