Recombinant Ebola virus nucleoprotein and glycoprotein (Gabon 94 strain) provide new tools for the detection of human infections

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After cloning and sequencing the glycoprotein (GP) gene of one of the Gabonese strains of Ebola virus isolated during the 1994–1996 outbreak, it was shown that the circulating virus was of the Zaire subtype. This was confirmed in this study by cloning and sequencing the nucleoprotein (NP) gene of this strain. These two structural proteins were also expressed as recombinant proteins and used in ELISA tests. NP was expressed as a His-tagged fusion protein in Escherichia coli and was purified on resins charged with nickel ions. GP was expressed by means of recombinant baculoviruses in Spodoptera frugiperda cells. Both recombinant proteins reacted positively in ELISAs for the detection of IgG antibodies in convalescent human sera from Gabon and Zaire. The difference in the relative titres of anti-NP and -GP antibodies was variable, depending on the sera. In addition, the recombinant NP reacted with heterologous sera from Côte d’Ivoire and was used successfully to detect IgM antibodies by μ-capture ELISA in sera from Gabonese patients.

Introduction

The two members of the family Filoviridae, Ebola and Marburg viruses, are responsible for severe forms of haemorrhagic fevers (Feldmann & Klenk, 1996; Peters et al., 1994). The Ebola virus species is split into four different subtypes called Zaire, Sudan, Côte d’Ivoire and Reston (Sanchez et al., 1996). The Zaire and Sudan subtypes were first isolated during the twin epidemics which occurred in 1976. The disease re-emerged in Zaire in 1977 and 1995, and in Sudan in 1979. The mortality rate reported during the outbreaks was 50–90%. The natural reservoir of the virus is still unknown. More recently, a new subtype, Côte d’Ivoire, was isolated from a young zoologist who became infected after post-mortem examination of wild chimpanzees (Le Guenno et al., 1995). The Côte d’Ivoire subtype of Ebola virus was found to be responsible for two outbreaks among the chimpanzee population of the Tai Forest region. The Reston subtype was isolated twice, in 1989 in USA and in 1992 in Italy, from cynomolgus monkeys imported from the Philippines and kept in quarantine (Jahrling et al., 1990). The Ebola virus Reston subtype appeared to be non-pathogenic for man. Recently, in 1994 and in February, March and July 1996, Gabon has endured three epidemics of Ebola haemorrhagic fever (Le Guenno & Galabru, 1997; Georges-Courbot et al., 1997). After isolation of the virus (Amblard et al., 1997), genomic sequence determination and phylogenetic analysis of the glycoprotein (GP) demonstrated that the Zaire subtype had re-emerged (Volchkov et al., 1997; Georges-Courbot et al., 1997).

Because of the severity of Ebola fever and its ability to be transmitted from man-to-man, suspected cases must be diagnosed as soon as possible after the onset of the disease. Isolation of the virus from blood samples is the method of choice but definite identification takes more than 1 week. A method based on the enzyme immunosorbent assay has been developed by Ksiazek et al. (1992) to detect antigens in patient samples. The effectiveness of the technique was tested during two outbreaks of Ebola infection among Macaca fascicularis monkeys kept in quarantine and it was recently used to detect human infections during the Zaire outbreak in 1995 (Ksiazek, 1996). Little is known of the immune response to Ebola virus infection but recent reports have indicated that antibodies can be detected as early as 4–5 days after the onset of disease (Ksiazek, 1996).

Several serological tests have been developed to detect antibodies: indirect immunofluorescence assay (IFA) using virus-infected cells; ELISA with antigens prepared by borate/
C. Prehaud and others

Triton X-100- extraction of the infected cells; Western blotting; and radio-immunoprecipitation (Ksiazek, 1991). Attempts to develop inhibition of haemagglutination and neutralization tests have failed due to the absence of haemagglutination activity in the virion and the absence or low level of neutralizing antibodies (Jahrling et al., 1996). Since these highly pathogenic viruses can be manipulated only under Biosafety Level 4 containment conditions and must be inactivated when used for diagnosis, recombinant antigens that are easy to prepare, safe for the manipulator and the environment, sensitive and reliable would be of considerable value.

The genome of filoviruses is composed of an RNA molecule of negative polarity (approximately 19 kilobases) encoding seven structural proteins in the order NP, VP35, VP40, GP, VP30, VP24 and L (Feldmann et al., 1992; Sanchez et al., 1993; Volchkov et al., 1992). Each protein is primarily synthesized from an individual polyadenylated mRNA. Transcription of the GP gene of Ebola viruses, however, leads to at least two different mRNAs. The primary transcript encodes a secreted non-structural small glycoprotein (sGP) and the envelope GP is expressed through transcriptional editing (Volchkov et al., 1992; Sanchez et al., 1996).

Becker et al. (1992) and Elliott et al. (1993) have reported that, by screening monkey and human sera for filovirus antibodies, the nucleoprotein (NP) and, in many cases, the structural proteins VP40, VP35 and VP30 were the major determinants in the immune response. In other forms of haemorrhagic fevers, i.e. those due to hantaviruses and to the arenavirus Lassa virus, the NP has been identified as a major antigen, inducing an early and long-lasting immune response (Barber et al., 1990; Lloyd et al., 1989; Zoller et al., 1993a,b).

Here, we have cloned, sequenced and expressed the NP of the Gabon 1994 strain of Ebola virus as a recombinant protein in bacteria. We also expressed the glycoproteins using recombinant baculoviruses in Spodoptera frugiperda (Sf9) cells and showed that, when used as antigens in ELISA tests, recombinant NP and GP reacted positively for the detection of IgG antibodies in convalescent human sera from Gabon and Zaire. In addition, the recombinant NP reacted with heterologous sera from Côte d’Ivoire and was used successfully to detect IgM antibodies in μ-capture ELISA. This work has demonstrated that recombinant NP and GP have properties similar to the authentic antigens in ELISA. Therefore, they represent a considerable source of antigens for the future.

**Methods**

**Virus and cells.** Vero E6 cells (ATCC #CRL-1586) cultivated in DMEM (Gibco/BRL) supplemented with 5% foetal calf serum were inoculated with a dilution 1:100 or 1:10 of the blood of patients suffering from a high fever and bloody diarrhoea in Gabon during the period from December 1994 to January 1995. The cell cultures were incubated at 37 °C for 14 days or until a clear cytopathic effect was observed. The maintenance medium was collected and served as a source of virus for further experiments. The remaining cells were washed with PBS and total RNAs were extracted using Trizol reagent (Gibco/BRL) according to the manufacturer’s instructions.

Nine isolates of the strain Gabon 94 were obtained and characterized by IFA and PCR amplification of a region located in the NP gene (Ambland et al., 1997). The molecular clones described here were obtained from a 1994 isolate designated Gabon 94.

**Cloning and sequencing of the Ebola virus NP gene.** Total RNA (5 μg) from cells infected with the Gabon 94 strains was denatured with methyl mercuro hydroxide (0·01 M). The RNA was subsequently complexed with β-mercaptoethanol and reverse transcribed with the M-MLV SuperScript reverse transcriptase (200 U; Gibco/BRL) for 2 h 45 min at 37 °C in a 20 μl reaction primed with the oligodeoxynucleotide E-Start (5’ GGCCTGATCAGGAGAAATTA 3’; the sequence of the restriction sites is underlined). Amplification by PCR was carried out in a 100 μl reaction with 5 μl cDNA, the oligodeoxynucleotides E-Init and E-Ter and 2·5 U Goldstar enzyme (5 μl; Eurogentec) under the conditions recommended by the supplier. The sequences of the oligodeoxynucleotides E-Init (5’ GGCCTGATCAGGAGAAATTAAAATTCGAGATAG 5’) and E-Ter (5’ GCCCTGATCAGGAGAAATTTCTTCCAAGCTCTATTTCA 5’) correspond to the 5’ and 3’ non-coding sequence of the NP gene of the Mayinga strain of subtype Zaire including the initiation or stop codon (in bold), respectively, preceded by the restriction sites HsdIII or BamHI (underlined). The PCR programme comprised four cycles (95 °C, 90 s; 37 °C, 30 s; 55 °C, 30 s; 72 °C, 5 min) followed by 30 cycles (95 °C, 90 s; 55 °C, 1 min; 72 °C, 5 min) and one final cycle (95 °C, 90 s; 60 °C, 1 min; 72 °C, 5 min).

The DNA fragment of the expected size (approx. 2200 bp) was purified with the Geneclean kit (BIO 101); digested with BamHI enzyme and cloned at the unique BamHI site of plasmid pGEM-4Z (Promega). Sequencing was performed by Sanger’s method with the T7 sequencing kit (Pharmacia).

**Expression and purification of the NP protein.** For expression in *Escherichia coli,* the BamHI insert was repaired in the Klenow fragment of DNA polymerase I and ligated into plasmid pET-14b (Novagen) that had previously been digested with NdeI, repaired with the Klenow enzyme and dephosphorylated with calf intestine alkaline phosphatase. The resulting plasmid, pET-N Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) to produce recombinant NP. Proteins were purified by affinity chromatography on a Nickel affinity column and eluted with 8 M urea as described earlier (Volchkov et al., 1992). The edited GP gene, containing eight adenosine residues encoding the full-length glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, pET-N.Ebola, was then used to transform bacteria Bl21 (DE3) (Novagen) into which the glycoprotein, was generated by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene) and the oligo-phosphate. The resulting plasmid, p
endonuclease HindIII. Positive clones were confirmed by sequencing using a cycle sequencing technique based on the dideoxy chain-termination method (ABI 377 automatic sequencer). Recombinant baculoviruses were produced after transfection of insect cells (Sf9) with the recombinant bacmids. Lysates from cells harvested 48 h post-transfection were tested for GP expression by immunoblotting using an anti-Ebola Zaire horse antiserum (dilution 1:3000) as described previously (Volchkov et al., 1995). Stocks of recombinant baculoviruses bac/sGP-7U and bac/GP-8U were prepared by infecting Sf9 cells with supernatants of transfected cells.

### ELISAs

ELISAs were performed as described by Prehaud et al. (1990) using the recombinant NP and GP or the authentic antigens. The latter consists of a 1:1000 dilution of a lysate of Vero E6 cells uninfected or infected with strain Gabon 94 as described previously (Le Guenno et al., 1995). Tests with recombinant NP were carried out with 50 ng purified NP (unless otherwise stated). Wells for the negative controls were coated with an equivalent fraction of proteins from lysates of bacteria harbouring pET-14b, purified by chromatography in the same way as NP. The eluted fraction contained trace amounts of proteins. Tests with recombinant GP contained the lysate from 2.5 \times 10^7 cells infected with GP-expressing baculovirus or with the wild-type baculovirus (negative controls).

After incubation with the diluted serum and extensive washing, peroxidase-labelled anti-human IgG (Sigma) was added at a 1:3000 dilution and the enzyme-linked secondary antibodies were detected with a tetramethyl benzidine peroxidase substrate/peroxidase solution (Kirkegard and Perry Laboratories).

### Detection of IgM by \(\mu\)-capture ELISA

The assay was performed as described by Zöller et al. (1993b). Briefly, the wells were coated with anti-human IgM antibodies (Sigma; dilution 1:2000) were incubated with human serum followed by the antigen (recombinant NP or Gabon 94-infected Vero cell lysate). Negative controls contained equivalent amounts of the purified fraction of bacteria harbouring the pET-14b plasmid or uninfected Vero cells. The antigen was then detected with an anti-Ebola Zaire virus rabbit serum and peroxidase-labelled anti-rabbit antibodies as described for conventional ELISA.

### Serum specimens

Several positive sera collected in Gabon (Amblard et al., 1997; Zaire (Yambuku in 1976; World Health Organization, 1978) and Côte d’Ivoire (Le Guenno et al., 1995) were selected to evaluate the recombinant proteins. The Gabonese sera Gab. 1, 3, 6, 8 and 12 were sampled during the acute phase of the illness (less than 1 month after the onset of the illness), whereas the others were from the late phase of the illness (more than 3 months after the onset of the illness). Negative sera, obtained from Africans living in the areas where the disease was identified, were included in the analyses.

### Results

#### Cloning, sequencing and expression of the NP gene of the Gabon 94 strain

The GP gene of several representatives of the four subtypes of Ebola virus, including the one from Gabon 94, has been sequenced (Georges-Courbot et al., 1997; Sánchez et al., 1996; Volchkov et al., 1995, 1997). In contrast, only the NP gene of the Mayinga strain (isolated in Zaire in 1976) was determined. Due to the extensive sequence homology in the extreme 5’ and 3’ non-coding regions of the GP genes, the primers for reverse transcription and PCR amplification of the NP gene were designed using the published sequence of the Mayinga strain (Sanchez et al., 1989). Amplification by RT–PCR resulted in the synthesis of a DNA fragment of the expected size (approx. 2200 bp) which was cloned into pGEM-4Z at the unique BamHI site. Two independent plasmids were sequenced and produced identical sequences (EMBL accession number Y09358). Comparison with the NP sequence of the Mayinga (Zaire 76) strain indicated divergence of 1.5 and 1.8% at the nucleotide and amino acid levels, respectively (Table 1), and confirmed that the Gabon 94 strain belongs to the Zaire subtype as already shown by sequencing the GP gene (Volchkov et al., 1997).

The complete NP gene was cloned into the pET-14b plasmid in-frame with the six histidine codons which represent the N-terminal sequence of the expressed fusion protein (Fig.

### Table 1. Nucleotide difference between the sequences coding for the NP protein of the Mayinga (Zaire 76) and Gabon 94 strains

Position 1 represents the A of the initiation codon.

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Expression of sGP and GP in Spodoptera frugiperda cells

The entire ORF of the Gabon GP gene was synthesized by RT–PCR and cloned into the pFastBac1 plasmid to produce a recombinant baculovirus bac/sGP-7U. In order to express the complete GP, site-directed mutagenesis was used to generate a mutant bac/GP-8U that carried eight consecutive adenosine residues at the editing site (Sanchez et al., 1996; Volchkov et al., 1995). Infection of Sf9 cells with bac/sGP-7U led to the expression of sGP (Fig. 2a), the primary gene product of the Ebola virus GP gene whereas, as expected, infection with bac/GP-8U produced GP (Fig. 2b). Small amounts of GP were detected following bac/sGP-7U infection, indicating that editing can also be mediated by the baculovirus RNA polymerase as demonstrated previously for vaccinia virus and T7 RNA polymerases (Volchkov et al., 1995). The sGP and GP synthesized in Sf9 cells migrated slightly faster than those expressed in mammalian HeLa cells in SDS–PAGE. This is most likely due to the difference in glycosylation. After infection with baculovirus, the recombinant protein was detected by ELISA using anti-Ebola and anti-GP antibodies.

Use of recombinant NP and GP as antigens for ELISA

Preliminary tests were conducted by coating wells with increasing amounts of recombinant proteins and using a positive serum at a 1 : 100 dilution. A strong signal ($A_{450} > 1$) was observed with 50 ng (or more) of the purified NP but this...
Recombinant Ebola virus proteins in ELISAs

Fig. 2. Expression of the Ebola Gabon strain sGP and GP in insect cells. Sf9 cells were infected with the recombinant baculoviruses bac/sGP-7U or bac/GP-8U at an m.o.i. of 1 p.f.u. per cell and lysed 48 h post-infection. Proteins were subjected to 15% (a) or 10% (b) SDS–PAGE and blotted onto nylon membranes. Immunoblotting was performed using an anti-Ebola Zaire serum. Lanes: a1 and b1, proteins of Ebola Zaire, strain Eckron, produced in the culture medium of infected Vero cells; a2 and a3, different amounts of lysates of bac/sGP-7U-infected Sf9 cells; b2, lysate of bac/GP-8U-infected Sf9 cells. GP and sGP, glycoprotein or small glycoprotein produced in mammalian cells; GP-bac and sGP-bac, glycoprotein or small glycoprotein produced in insect cells; asterisk in a3, small amounts of GP generated by RNA editing.

Fig. 3. Comparison of the authentic antigen and the recombinant NP and GP in ELISA test for IgG detection. (a) IgG reactivity of human sera from Zaire (Z.) or Gabon (Gab.) assayed by ELISA at a 1 : 100 dilution. An African serum (SA. 74) was run as a negative control in this experiment. Recombinant NP protein, lysate from Sf9 cells expressing GP or the reference antigen were used to coat the wells. (b) Four of these sera were titrated for IgG detection by ELISA after serial 3-fold dilutions using the recombinant NP, baculovirus-infected Sf9 cells expressing GP or the reference antigen.

was reduced to the background level with 12 ng protein. It was then decided to coat the wells with a standard amount (50 ng). For the negative controls, the wells contained an equivalent fraction of proteins from bacteria harbouring the pET-14b plasmid.

Similarly, we established that wells coated with proteins from 2.5 × 10³ Sf9 cells expressing GP gave an optimal response (data not shown). Negative controls consisted of wells coated with lysates of the same number of cells infected with the wild-type baculovirus. In parallel experiments, other plates were coated with the authentic antigen prepared from Vero cells infected with Ebola Gabon 94 virus. Uninfected cell extracts served as negative controls.

Four convalescent sera from the epidemics of Yambuku in 1976 and three from Gabon in 1994 were first tested at a 1 : 100 dilution (Fig. 3a). Clearly IgG antibodies specific for NP and GP were detected in all the sera. To evaluate the sensitivity of the ELISA based on the recombinant antigens, titration curves were determined and compared with those of the authentic antigen. Fig. 3(b) illustrates the results obtained with
some of the positive sera. The reference antigen which comprises all the viral proteins exhibited a sigmoid curve in most cases and produced the highest antibody titre. When assayed independently with recombinant proteins, titres of some of the sera, such as Zaire 76-1 and 76-3, obtained with the GP antigen were much lower than those determined with the recombinant NP whereas in others (Zaire 76-4 and Gabon 24) the anti-GP and NP antibody titres were not significantly different. All the Zaire and Gabon 24 sera were collected late after infection (more than 3 months after the onset of disease) whereas Gabon 1 and 12 were collected during the acute phase (less than 1 month after the onset of the disease). Although a relatively low number of sera were tested in these experiments, no correlation could be established between the relative amount of antibodies against NP and GP and the stage of infection.

To evaluate the validity of the recombinant antigens in diagnostic tests, 24 African sera from Côte d’Ivoire were tested. Twenty-two of these sera had been determined as negative by ELISA using the conventional antigen and by indirect IFA on acetone-fixed Ebola virus (Gabon subtype)-infected Vero E6 cells; the remaining two sera tested positive in the same tests and were added as positive controls. In all the ELISA tests, the signal was calculated as the difference between $A_{450}$ obtained with the Ebola virus-infected and uninfected cell extracts, or with NP and the negative control protein or that with GP-expressing Sf9 cell lysate and wild-type baculovirus virus-infected cell extracts. All the negative sera gave a low absorbance with the reference antigen (mean $A_{450}$ $0.09 \pm 0.09$) and with NP (mean $A_{450}$ $0.07 \pm 0.06$); P/N ratios ($A_{450}$ measured in the positive well/$A_{450}$ in the background well) were 2.5 and 8, respectively. For the two positive sera, this ratio was calculated to be 20 and 35 with the reference antigen and the recombinant NP, respectively. These results indicate that the test using this recombinant protein is highly specific. When tested for anti-GP antibodies, the negative sera produced relatively high and variable absorbance values in both the test well (mean $A_{450}$ $0.49 \pm 0.36$) and in the background well (mean $A_{450}$ $0.29 \pm 0.36$). As for the positive sera, they gave clear positive results but the P/N ratio was close to 2 indicating that, although the crude GP-expressing Sf9 cell lysate is a valuable antigen to determine the presence of anti-GP antibodies in positive sera, the high background absorbance jeopardizes the use of crude GP-expressing Sf9 lysates for routine screening in serosurveys. This problem, a consequence of using insect cells that is not encountered with mammalian cells, may be solved by expressing a His-tagged GP protein which could be easily purified.

Since viral NPs are thought to be more conserved, the reactivity of the recombinant NP with heterologous sera was assessed. Thus, we tested the sera from the patient infected with the Côte d’Ivoire subtype and collected during convalescence (IC 95) or more than one year after recovery (IC 96). For comparison, three additional Gabonese sera were assayed in parallel. All the sera reacted positively with the recombinant NP and the titres determined with the recombinant protein and the reference antigen were in the same range (Fig. 4). Specific sera for the Reston and Sudan subtypes, unfortunately not available for this study, should also be tested to determine whether the recombinant Gabon NP has a broad specificity.

**Detection of IgM antibodies by µ-capture ELISA**

Since the presence of IgM antibodies is a sign of early infection, we assessed whether the purified bacterial fusion protein could favourably replace the reference antigen in a µ-capture ELISA test. Except for the serum Gabon 24, which was collected one year after remission, we selected and tested several sera, Gabon 3, 6 and 8, collected during the acute phase of the disease (i.e. less than one month after the onset of the disease). Sera IC96 and Zaire 76-3, collected during the late phases of disease, were included as controls. When the authentic and recombinant NP antigens were compared for their sensitivity towards the IgM antibodies, all the sera which reacted positively with the conventional antigen were positive with the recombinant NP. Titration curves (Fig. 5) clearly indicate that NP induced an early humoral response in humans.
infected with Ebola virus. As only a few sera collected during the acute phase of the disease were still available, it was not possible to test the performance of this assay with heterologous strains.

**Discussion**

Compared with the viral antigens prepared from highly pathogenic viruses such as filoviruses, which must be manipulated in a Biosafety Level 4 facility, the recombinant proteins obviously offer two main advantages: they are safe and easy to prepare and the amount of antigen used in the assays can be easily standardized.

Several recombinant NPs of haemorrhagic fever viruses have been produced by using bacterial or baculovirus expression systems (Barber et al., 1990; Gött et al., 1991; Kallio-Kokko et al., 1993; Zöller et al., 1993a,b). These recombinants were efficiently recognized by patient sera, but had a tendency to form aggregates or to be insoluble, which jeopardizes the reproducibility of results with batches. The Ebola virus His-tagged NP protein that we expressed in this study was mostly produced as a soluble protein and in relatively large quantities. Several batches of protein were produced and reproducible ELISA data were obtained with a dose of 50 ng antigen per coated well.

Since filovirus GPs are highly glycosylated, containing both N- and O-linked carbohydrates which contribute to one-third to one-half of their molecular mass (Feldmann et al., 1991, 1994), we expressed the Ebola virus GP in the eukaryotic baculovirus system rather than in the E. coli system, which lacks glycosylation ability. Significant amounts of sGP and GP were synthesized in SF9 cells and were immunoreactive with anti-Ebola virus antibodies. Crude lysates of insect cells infected with baculovirus expressing the complete GP form were analysed further.

The data reported in this study indicated that IgG antibodies against the recombinant NP and GP were detected in all the sera from patients who had suffered from Ebola fever. The difference in the relative titres of anti-NP and -GP antibodies could be due to the individual immune response of the patients or the stage after infection when the sera were collected. Although all the sera from patients clinically and serologically diagnosed with Ebola fever had antibodies against NP and GP, other structural proteins might also act as important immunogens. This has been documented by Becker et al. (1992) and Elliott et al. (1993) who screened sera from monkeys and humans by Western blotting and showed that NP, VP40, VP35 and VP30 were frequently detected.

Whereas detection of IgG antibodies is the test of choice for large screening of serological surveys, detection of IgM is a useful method for early diagnosis. The recombinant NP has proved to be useful in the assays reported here, using homologous sera collected from Gabonese patients during the early phase of the disease. Unfortunately, early sera from Côte d’Ivoire were not available when this test was set up. If this assay could also detect heterologous IgM, it could be used in parallel with the antigen-capture assay described by Ksiazek et al. (1992). Practically, both of these assays have their limits and other tests such as virus isolation or RT–PCR amplification for genome detection could be used for confirmation.

An important drawback in filovirus detection is the high amount of false positives determined in serosurveys (Peters et al., 1994). The use of the purified recombinant NP and GP in ELISA tests should help to solve this problem. Provided that the filovirus reservoir is performed by screening the collected samples for antibodies, ELISA tests using recombinant antigens will be useful.

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**References**


