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RESEARCH ARTICLE

Comparative sequence analysis and expression in *E. coli* of the subgroup I-specific antigen VP6 from a G2 serotype human rotavirus IS2

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VP6, the intermediate capsid protein of the virion, specifies subgroup specificity of rotavirus. It is also the most conserved, both at nucleotide and amino acid levels, among group A rotaviruses and is the target of choice for rotavirus detection. In this study we report the sequence of the subgroup I (SGI)-specific VP6 from the serotype G2 strain IS2 isolated from a child suffering from acute diarrhoea in Bangalore and its comparison with the published VP6 sequences. Interestingly, IS2 gene 6 shared highest homology with that from bovine UK strain and the protein contained substitutions by lysine at amino acid positions 97 and 134. In contrast, the amino acids Met and Glu/Asp at these respective positions are highly conserved in all the other group A rotaviruses sequenced so far. These observations have obvious implications for the evolution of serotype G2 and G2-like strains circulating in India. The SGI VP6, of a human rotavirus, possessing epitopes that are conformationally similar to those found in the native protein in the virion, was successfully expressed in *E. coli* and purified for the first time by single-step affinity chromatography.

DIARRHOEAL diseases are the major cause of morbidity and mortality among infants and young children, especially in developing countries. Of the many diarrhoeal agents, rotavirus is the single most important cause of severe, acute infantile gastroenteritis in humans and a variety of domestic animals[1]. Rotavirus diarrhoea occurs throughout the year and is estimated to account for about a million deaths annually among young children, thus representing an important public health problem[1,2]. In spite of the staggering economic burden, an effective vaccine against rotavirus diarrhoea is yet to be developed.

Rotavirus belongs to the family Reoviridae and consists of a triple-layered capsid[3]. The outer shell consists of two proteins, VP4 and VP7, the intermediate shell comprises of VP6 and the inner shell is composed of VP2 that encloses a genome of 11 segments of double-stranded (ds) RNA[3,4]. The genome encodes 6 structural

and at least 5 nonstructural proteins[5]. The outer capsid proteins VP7 and VP4 specify two independent serotype specificities, the G and P types respectively[6]. VP6, encoded by gene segment 6, contains group as well as subgroup-specific epitopes[6,7]. On the basis of group-specific epitopes, 7 groups A to G have been identified in humans and animals[5]. Group A rotaviruses, the most common pathogens in humans, can be further subdivided into at least 4 subgroups SGI, SGII, SGI + II and non SGI/II on the basis of the SG-specific epitopes[8]. Group A rotaviruses can also be initially characterized as 'short' or 'long' electropherotypes depending on the slower or faster electrophoretic migration, respectively of the dsRNA segment 11 in polyacrylamide gels[1]. In general, majority of the human rotaviruses with SGI specificity belong to G2 serotype and exhibit 'short' RNA electropherotype whereas those with SGII specificity have 'long' RNA pattern and belong to other serotypes[1]. In contrast, the great majority of animal strains appear to possess 'long' RNA pattern but SGI specificity[1,9].

VP6 comprising more than 50% of the virion mass is an important viral antigen and is involved in several viral functions such as replication, transcription and viral morphogenesis[5,8,10,11]. Although majority of the serum antibodies in the infected host are directed against VP6, anti-VP6 IgG antibodies have not been conclusively shown to be capable of virus neutralization[8]. Recent studies, however, indicate that mucosal anti-VP6 secretory IgA antibodies play an important role in protection against the rotavirus disease[12]. VP6 also contains epitopes recognized by cytotoxic T-lymphocytes as well as helper T cells, indicating a role for VP6 in stimulating heterotypic cell-mediated immune response[13–15]. VP6 was shown to function as an excellent immunological carrier for peptides and proteins in vaccine development[16]. Because of its high abundance, stability and high degree of conservation among human and animal group A rotaviruses, majority of the procedures for detection of rotaviruses in clinical samples are based on VP6 (refs 7, 17, 18).

Although the SGI-VP6 from a few animal strains had been expressed[19–12], that from a human rotavirus has not been reported. Because of the importance of VP6 in viral diagnosis and to determine the sequence variation in VP6 from Indian rotaviruses, we report here the cloning, comparative sequence analysis and expression in *E. coli* of the SGI VP6 from an Indian G2 serotype strain IS2 isolated from a child with diarrhoea in Bangalore.

Materials and methods

Extraction of viral genomic dsRNA and purification of the RNA segments

Isolation and serotypic characterization of rotaviruses including the G2 serotype strain IS2 isolated from children suffering from diarrhoea admitted to various hospitals in Bangalore has been described earlier[18]. As IS2 was not adapted to growth in culture, nucleic acids were extracted directly from the clarified supernatants of the 20% suspension of the stool sample as previously described[18]. The dsRNA was electrophoresed on a 1% agarose gel in presence of ethidium bromide and the RNA segments 5, 6, 7, 8 and 9 were electroeluted together onto a dialysis membrane and purified by phenol–chloroform extraction[22]. For identification of gene 6-specific cDNA clones, gene 6 RNA segment was separately purified from the agarose gel.

cDNA synthesis, cloning and identification of VP6 gene-specific clones

In vitro polyadenylation of the denatured dsRNAs, synthesis of cDNA on the oligo(dT)-tailed plasmid (pCDV) primer, construction of cDNA library in *E. coli HB101* were described previously[22–25]. The cDNA library, constructed for segments 5 to 9, was screened with ^{32}[P]-labelled mixed cDNA probe for segments 5, 6, 7, 8 and 9. Gene 6-specific cDNA clones were identified by Southern blot hybridization of the BamHI-digested plasmid DNAs with either the cDNA probe prepared from purified RNA segment 6 or the RNA probe prepared by labelling at the 3′ end using *E. coli* poly(A)-polymerase[25]. Several clones containing inserts ranging in size from 600 to 1500 nucleotides (nt) were identified. Clones OB67 having an insert of 1.0 kb and OB48, OB49 and OB68 containing inserts of 1.5 kb were used for further analysis. Although the reported length of gene 6 from group A rotaviruses is 1356 nt, the observed size of 1.5 kb can be attributed to the presence of poly(dA) and poly(dG)-tails of variable length at the 3′ and 5′ ends of the cDNAs respectively.

Nucleotide sequence analysis

The complete nucleotide sequence of gene 6 was determined from partial and full length cDNA clones. The cDNA inserts from the original clones were subcloned at BamH I site of pBluescript KS$^+$ (pBSKS$^+$) vector (Stratagene, CA, USA). From pBS67, subclones were generated utilizing internal sites for restriction endonucleases Nhe I, Pst I and Xba I. Sequence of both strands of the inserts in the subclones was determined using KS, SK and gene-specific primers. Nucleotide sequence was determined by dideoxynucleotide-mediated chain termination method[26] using sequenase version 2.0. Sequence near the 5′-end of the inserts in the original clones was determined using Okayama and Berg vector-specific primer. Nucleotide and the deduced amino acid sequences were analysed using version 6.1 of the GCG application software at the Distributed Information

Centre, Indian Institute of Science. The sequences of the oligonucleotide primers are: 5′-ATCACAACCAGCT CATGAT-3′ from nt position 527 to 545; 5′-TTAACTACAGCTACAAT-3′ from nt position 675 to 691; 5′-GAAGTGTTACTTCTGCTCT-3′ (5′ primer for Okayama and Berg vector).

Expression in E. coli and purification of VP6

For VP6 expression, the T7-promoter – and the polymerase – based expression system was used[27]. As the complete gene 6 cDNA contained 5′ and 3′ untranslated sequences, the gene 6 ORF was amplified by polymerase chain reaction (PCR)[28] using primers corresponding to the 5′ and 3′ ends of the ORF. The primers contained sites for restriction enzymes of convenience. The sequence of the 5′ primer is 5′-GATATCAAGCTT CCCGGGATGGATGTCCTGTACTC-3′ and that of the 3′ primer is 5′-CTGCAGAAGCTTTTTGACAAGCAT GCTTCT-3′. Gene 6 specific sequence is underlined. The PCR-amplified DNA was digested with Hind III and inserted at the Hind III site of pET20b(+) vector[27] (Novagen, Madison, WI, USA). The resulting construct is named as pETG6. To express VP6 without the pelB leader sequence, pETG6 was digested with Nde I and Sma I and religated after blunting the Nde I end by Klenow fill-in reaction. Deletion of the pelB leader sequence as well as the stretch of 17 aa downstream of it (upstream of the gene 6 AUG codon) brings the gene 6 AUG codon to within the requisite distance (10 nt in this case) from the Shine-Dalgarno (SD) sequence[29]. This construct was represented as pETNDG6. *E. coli HB101* cells were transformed with the ligated DNA and the plasmid DNAs from positive clones were used to transform *E. coli* BL21 (DE3)[27]. Expression of VP6 in pETG6 and pETNDG6 recombinants was examined by analysis of the cell lysates by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described[30]. The recombinant VP6 was purified by single-step affinity chromatograpy using Ni^{2+}-NTA-agarose as described earlier[24].

Production of polyclonal antiserum and immunoblot analysis

About 500 µg of the recombinant VP6 protein purified from the Ni^{2+}-NTA-agarose column was electrophoresed on a 12% preparatory SDS-polyacrylamide gel. The 46.4 kDa band corresponding to VP6 was excised after staining with Coomassie blue and homogenized in 1 ml of phosphate-buffered saline. Polyclonal antibodies were raised by injecting the gel suspension into a New Zealand white rabbit subcutaneously at multiple sites as previously described[22]. Pre-immune serum collected from the same rabbit before immunization was used as control.

Purity of the column-purified recombinant VP6 as well as the specificity of the polyclonal antiserum was determined by immunoblot analysis of the protein[31]. Western blots containing either the total cell lysate or the affinity-purified protein were first incubated with either the anti-VP6 antiserum or the SGI-specific mAb 255/60 and then with the secondary antibodies, goat anti-rabbit IgG or goat anti-mouse IgG conjugated with the horse radish peroxidase, respectively. For detection of VP6 by the SGI mAb, the gel was soaked in renaturation buffer (50 mM Tris.Cl pH 7.4, 20% glycerol) for 20 min prior to blotting onto nitrocellulose membrane. The antigen antibody interaction was detected by colour development in citrate buffer containing 3,3-diaminobenzidine tetrahydrochloride and H_2O_2. The SGI-specific mAb 255/60 was originally produced using rhesus rotavirus (RRV) as the immunizing virus[7] and provided by Dr Harry B. Greenberg, Stanford University, USA.

Immunoprecipitation

Individual colonies of BL21 (DE3) containing either pETG6 or pETNDG6 were grown overnight in M9 medium. The cultures were then inoculated into fresh sulfate-free M9 medium at 100-fold dilution and grown until the OD_{600} reached 0.4. The cells were then induced with 0.4 mM IPTG in presence of 10 µCi of ^{35}[S]-methionine per ml for 10 min at 37°C. The cells were harvested, lysed and inclusion bodies were prepared as described earlier[24]. The inclusion bodies were dissolved in 0.1 M Tris.Cl pH 8.5 buffer containing 8 M urea by incubating at room temperature for 30 min. Urea was removed from the lysate by centrifugation through a centricon 30 column at 5000 rpm for 30 min. The protein solution remaining in the column was diluted with a buffer containing 10 mM Tris.Cl pH 8.0 and 100 mM NaCl. The process was repeated 3 to 4 times and the final fraction remaining in the column was used for immunoprecipitation. About 50 µl of the protein solution was diluted to 300 µl with RIPA buffer and incubated on ice with 5 µl of mAb 255/60 for 1 h after which 200 µl of protein A-sepharose CL-4B suspension (5 mg of dry gel) was added. The radioactively labelled recombinant VP6 bound to the resin was analysed on a 12% SDS-polyacrylamide gel and the bands were detected by autoradiography as previously described[32].

Results

Nucleotide sequence analysis of gene 6 from the symptomatic G2 serotype Indian strain IS2 revealed that the

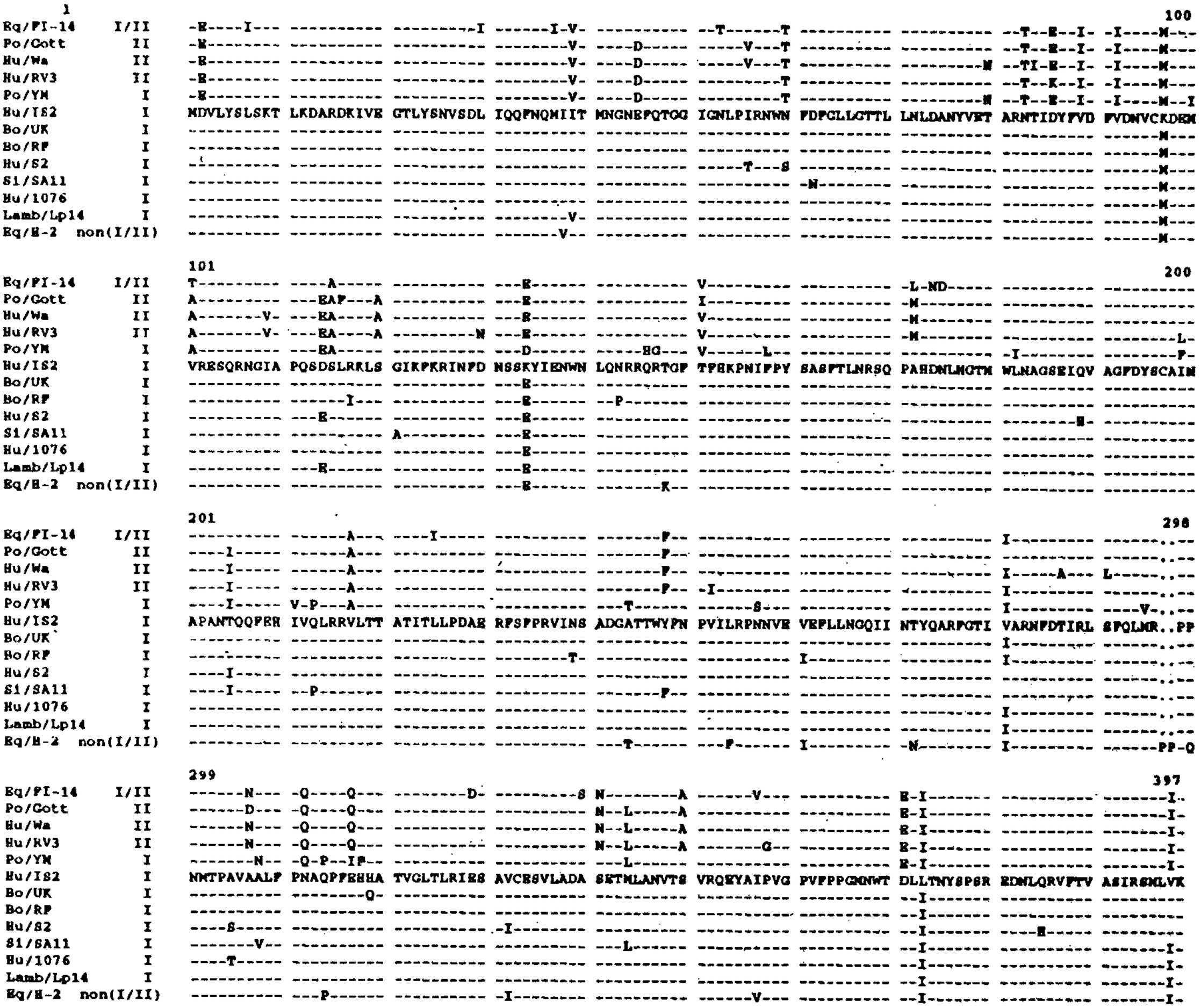

Figure 1. Comparison of the deduced amino acid sequence of IS2 VP6 with VP6 from other group A rotaviruses. Species origin of the rotavirus strains and the SG specificity of each of the strains are indicated on the left side of the corresponding VP6 sequence. Only the aas that differ from IS2 VP6 are shown. The numbering corresponds to the VP6 from majority of the strains that contain 397 aas. VP6 from the equine strain H2 is 399 aas in length and contains a two-aa insertion at position 296. The IS2 gene 6 sequence was submitted to EMBL database with accession number X94617.

Table 1. Per cent nucleotide and amino acid sequence identities of IS2 VP6 with VP6 from other group A rotaviruses

Rotavirus strain	Species origin	SG	nt	aa
IS2	Human	I	–	–
UK	Bovine	I	94.48	98.74
RF	Bovine	I	93.84	97.73
SA11	Simian	I	87.30	97.23
S2	Human	I	86.74	97.23
1076	Human	I	86.60	98.49
LP14	Lamb	I	85.51	98.24
H2	Equine	nonI/II	82.31	96.47
FI-14	Equine	I/II	79.92	91.44
Gottfried	Porcine	II	79.82	92.44
Wa	Human	II	79.10	91.18
YM	Porcine	I	78.78	90.43

gene was 1356 nt in length. The gene contained a 5′ untranslated region (UTR) of 23 nt followed by a long ORF with an AUG codon from position 24 to 26 and a termination codon from position 1215 to 1217. The ORF is followed by a 3′ UTR of 139 nt. The ORF codes for a polypeptide of 397 aa with an apparent molecular weight of 44.87 kDa which is similar to that from other group A rotaviruses[5] (Figure 1). The initiation codon at nt position 24 has the optimal sequence context for a strong initiation codon[33]. There is no polyadenylation signal, AAUAAA, in the 3′ UTR which is a characteristic of the genomes of the members of the family Reoviridae[34].

Comparison of the nt and the deduced aa sequences of gene 6 of IS2 with those of other strains showed a high

degree of conservation of the SGI VP6 from different strains (Figure 1). The homologies for SGI VP6 ranged from 94.5% to 85.51% at the nt level and 98.74% to 97.23% at aa level (Table 1) with the exception of the porcine Ym VP6. The nt percentage homology with SGII VP6 from different strains was significantly low. Interestingly, IS2 gene 6 shared greatest sequence identity at both nucleotide and amino acid levels (94.48 and 98.74%, respectively) with that from the UK strain of bovine rotavirus and showed less identity (86.74% at nt and 97.23% at aa levels) with the SGI VP6 from the G2 type human strain S2 (Table 1). Majority of the prolines and the three cysteines at positions 96, 197 and 331 are conserved in VP6 belonging to all the subgroups indicating their importance in the proper folding of the protein. The Ile at aa position 56 and Ser at 120 that are conserved in all the SGI and SGI/II VP6 proteins are believed to contribute to SGI specificity[35]. Ala at positions 172 and 305 that determine SGI specificity are also conserved[36] in IS2 VP6. The aa sequences from 58 to 62 (NWNFD) and 159 to 165 (PYSASFT) that determine group-specific epitopes are also conserved[37] in IS2 VP6 (Figure 1).

The most striking observation was that the IS2 VP6 contained substitutions by lysine at aa positions 97 and 134 (Figure 1). In all other strains, irrespective of the subgroup, Met at 97 and Glu/Asp at 134 are highly conserved[35,38] (Figure 1). Also, IS2 VP6 contained a Leu at position 371 in contrast to Ile present at this position in all other group A rotaviruses (Figure 1). The isoelectric point of IS2 VP6, as determined by the programme ISOELECTRIC, was found to be more basic pI (6.68) compared to that of the VP6 from all other group A rotaviruses which ranged from 5.50 to 6.08 with the sole exception of the SGII VP6 from RV3 which had a pI of 7.03.

VP6 was expressed at high level in *E. coli* BL21 (DE3) using the pET20b(+) vector. While an expected 46.4 kDa band was detected in cells transformed with pETNDG6, two polypeptides of molecular weight 50.4 kDa and 48.0 kDa were observed in cells transformed with pETG6 (Figure 2). pETNDG6 lacks the pelB leader as well as the stretch of vector sequence encoding 17 aas preceeding the HindIII site in the vector. Thus the two polypeptides expressed from pETG6 might represent the leader-uncleaved and leader-cleaved forms of the VP6. It appears that the system is overburdened with the recombinant protein and only a fraction of the expressed protein underwent cleavage liberating the 48 kDa VP6 that contained the carboxyterminal six histidines. Inefficient cleavage of the pelB leader has also been observed previously by others[39]. Alternatively, the 48 kDa species could represent VP6 initiated at an internal AUG codon, most likely that at the NcoI site located downstream of the pelB leader sequence. The fact that only the expected 46.4 kDa protein was expressed from pETNDG6 in the absence of the pelB leader sequence strongly suggests that the 48 kDa protein expressed from pETG6 is derived from the 50.4 kDa precursor by cleavage of the leader sequence. The 46.4 kDa protein represented about 12% of the total protein. Clear mobility differences between the 48 kDa and the 46.4 kDa bands could not be detected in this size range in the mini gels used for electrophoretic separation (Figure 2) but mobility differences could be detected when electrophoresis is continued for longer period.

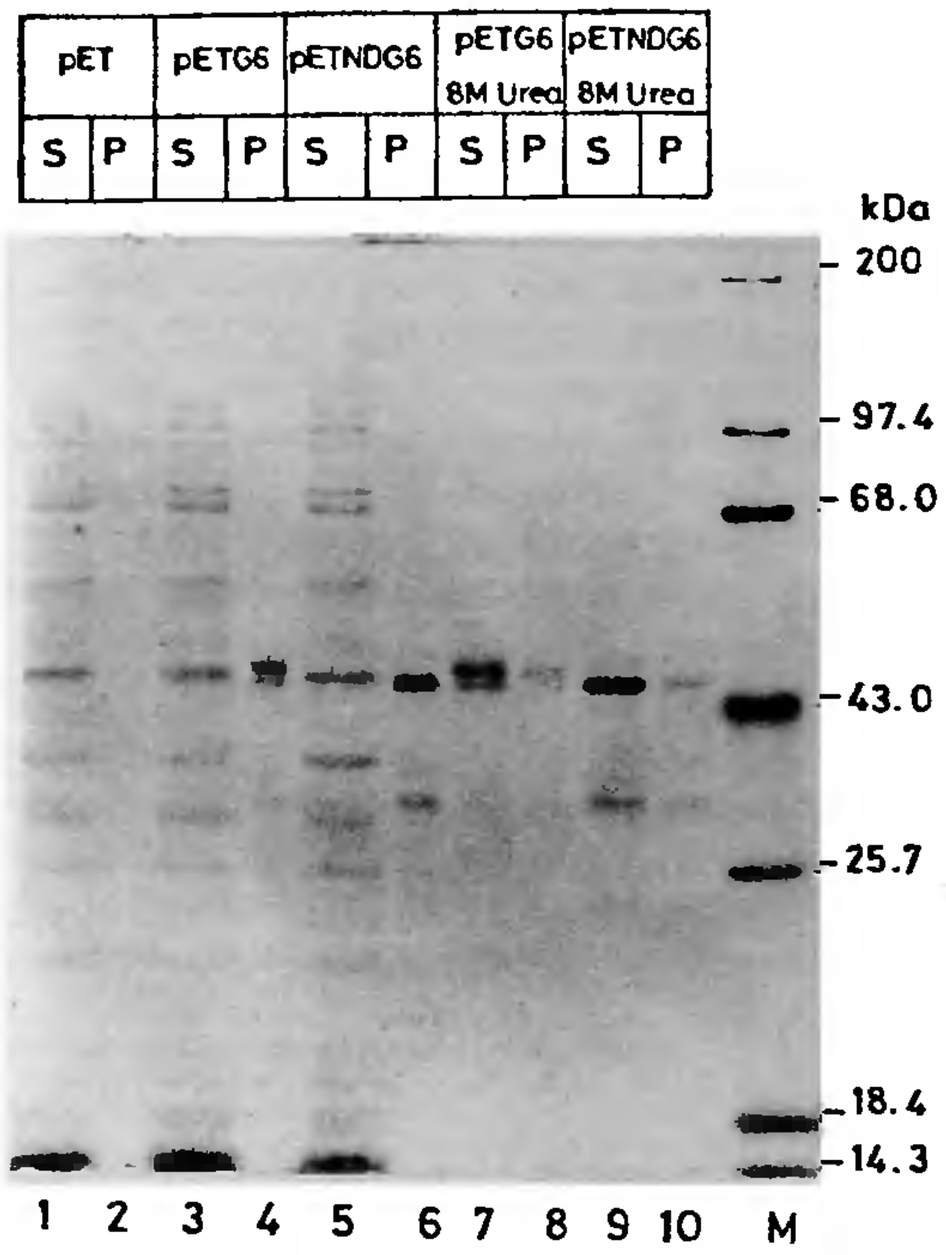

Figure 2. Expression of IS2 VP6 from pETG6 and pETNDG6 in *E. coli*. Soluble fractions of the total cell lysates from cells containing pET, pETG6 and pETNDG6 (lanes 1, 3 and 5, respectively) and the corresponding insoluble fractions (inclusion bodies) of the lysates (lanes 2, 4 and 6) were analysed by SDS-PAGE and the gel was stained with Coomassie blue. Lanes 7 and 9 contain VP6 extracted from the inclusion bodies in 8 M urea and lanes 8 and 10 represent the urea-insoluble fraction. M, protein molecular weight markers. Note that the recombinant VP6 was found only in the insoluble fraction at any time after induction with IPTG.

The recombinant protein expressed from either pETG6 or pETNDG6 and purified by affinity chromatography using Ni^{2+}-NTA-agarose resin, upon SDS-PAGE and staining with either Coomassie blue or silver nitrate showed another band of approximately 34 kDa (Figure 3, lane 3 and Figure 4). Silver staining of the gel also revealed another band of approximate size of 24 kDa (Figure 3). These low molecular weight species represent the carboxy terminal half of the protein as they

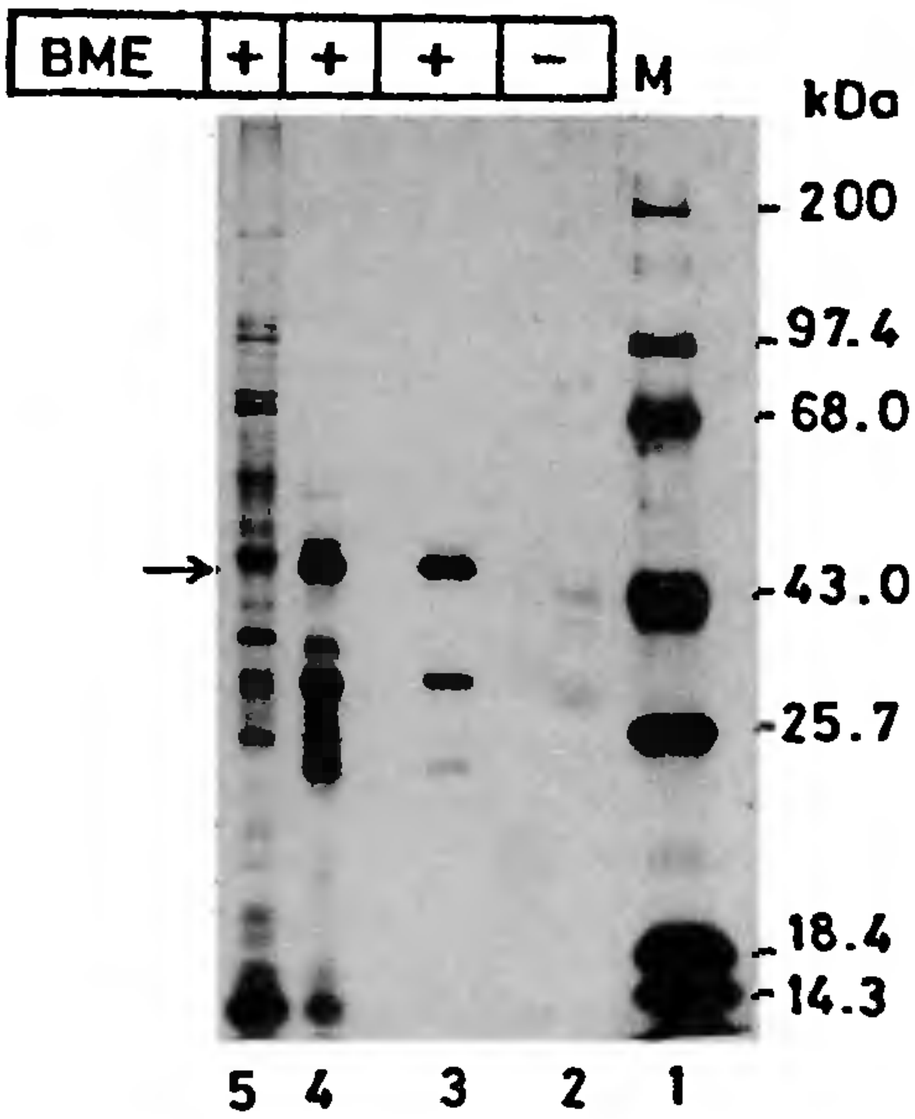

Figure 3. VP6 expressed in *E. coli* forms oligomeric structures. VP6 from the inclusion bodies was solubilized in 8M urea and the urea was then removed by gradient dialysis in presence (lane 3) or absence (lane 2) of β-ME. The proteins were separated by SDS-PAGE after heating at 100°C for 10 min in sample buffer containing (lane 3) or lacking (lane 2) β-ME. The gel was stained with silver nitrate. Lane 4 represents total insoluble fraction and lane 5 contains soluble fraction of the lysate from cells containing pETNDG6. Faint bands of high molecular weight forms of VP6 are seen in lane 2. Also the 24 kDa band is clearly seen in the reduced sample upon silver staining.

contained the histidine tag and could have arisen due to proteolytic cleavage or by internal initiation of translation at Met codons located at nt positions 321 and 552 or 561, respectively.

VP6 expressed in *E. coli* formed inclusion bodies as soon as it was synthesized, probably due to its intrinsic property to form multimeric structures. Studies on induction for varying time periods and with different concentrations of IPTG (0.1 μM to 0.4 mM) revealed that VP6 expressed at as early as 1 to 10 min of induction was present only in the insoluble fraction. At no stage could we detect VP6 in the soluble fraction of the cell lysate even by immunoblot analysis using polyclonal antiserum.

VP6 from inclusion bodies was examined for oligomeric association and for the presence of the conformation-dependent SGI-specific epitopes. VP6 from the inclusion bodies was solubilized in presence of 8 M urea and the protein was allowed to refold by gradual removal of the urea by dialysing against buffer containing successively decreasing concentration of urea. The soluble protein so obtained was subjected to SDS-PAGE in presence or absence of β-ME (Figure 3). In the absence of reducing agent, faint bands of higher molecular weight forms corresponding to dimers and trimers could be seen even after heating at 100°C upon silver staining (Figure 3, lane 2), indicating that VP6 expressed in *E. coli* forms oligomeric structures. When the same was not heated (in the presence or absence of β-ME), much of the protein appeared as a smear in the size range between 150 and 80 kDa (data not shown). But upon heating (lane 2) in the absence of β-ME, much of the protein dissociated into the monomer though significant amount also remained in the oligomeric forms. These results suggest that VP6 expressed in *E. coli* existed in different conformational states probably due to rapid formation of insoluble complexes. As observed in the native viral protein and that expressed in insect cells[40], the recombinant VP6 exhibited anomalous mobility (smaller than the expected 46.4 kDa) as well as heterogeneity in the 46.4 kDa region in the absence of β-ME, suggesting the presence of intramolecular disulphide bridges within the monomer (Figure 3, lane 2).

To determine the authenticity of the protein as well as the presence of the conformation-dependent subgroup I-specific epitopes on the recombinant protein, total lysates in Laemmli buffer were subjected to SDS-PAGE and immunoblot analysis. As shown in Figure 4 B, the recombinant protein was recognized by the SGI-specific mAb 255/60, indicating that the recombinant VP6 contained epitopes that are conformationally similar to those of the native protein in the virion. This is further substantiated by immunoprecipitation of the solubilized radioactively labelled protein with the SGI mAb 255/60 (Figure 4 C). Both polyclonal and monoclonal antibodies recognized the 50.4 kDa, 48.0 kDa, 46.4 kDa and 34 kDa polypeptides while the 24 kDa species could not be readily detected, probably reflecting a lack of all the regions that determine the SGI epitope in this polypeptide (Figure 4 A and 4 B). The intensity of the VP6 band observed in immunoblot and immunoprecipitation analyses (Figure 4, panels B and C) using the SGI-specific mAb 255/60 was not commensurate with the amount of protein used for analysis, reflecting that only a fraction of the VP6 contained conformational epitopes similar to those observed in the native protein.

Discussion

As part of our ongoing studies on the genetic and antigenic variation/diversity in rotaviruses circulating in Indian population, we determined the nucleotide sequence of the SGI-specific intermediate capsid protein VP6 from an Indian strain of symptomatic human rotavirus IS2. As observed in other rotaviruses, the VP6 gene from IS2 is also highly conserved. Comparative sequence analysis revealed that the IS2 VP6 shared greatest sequence identity with the SGI VP6 from the bovine

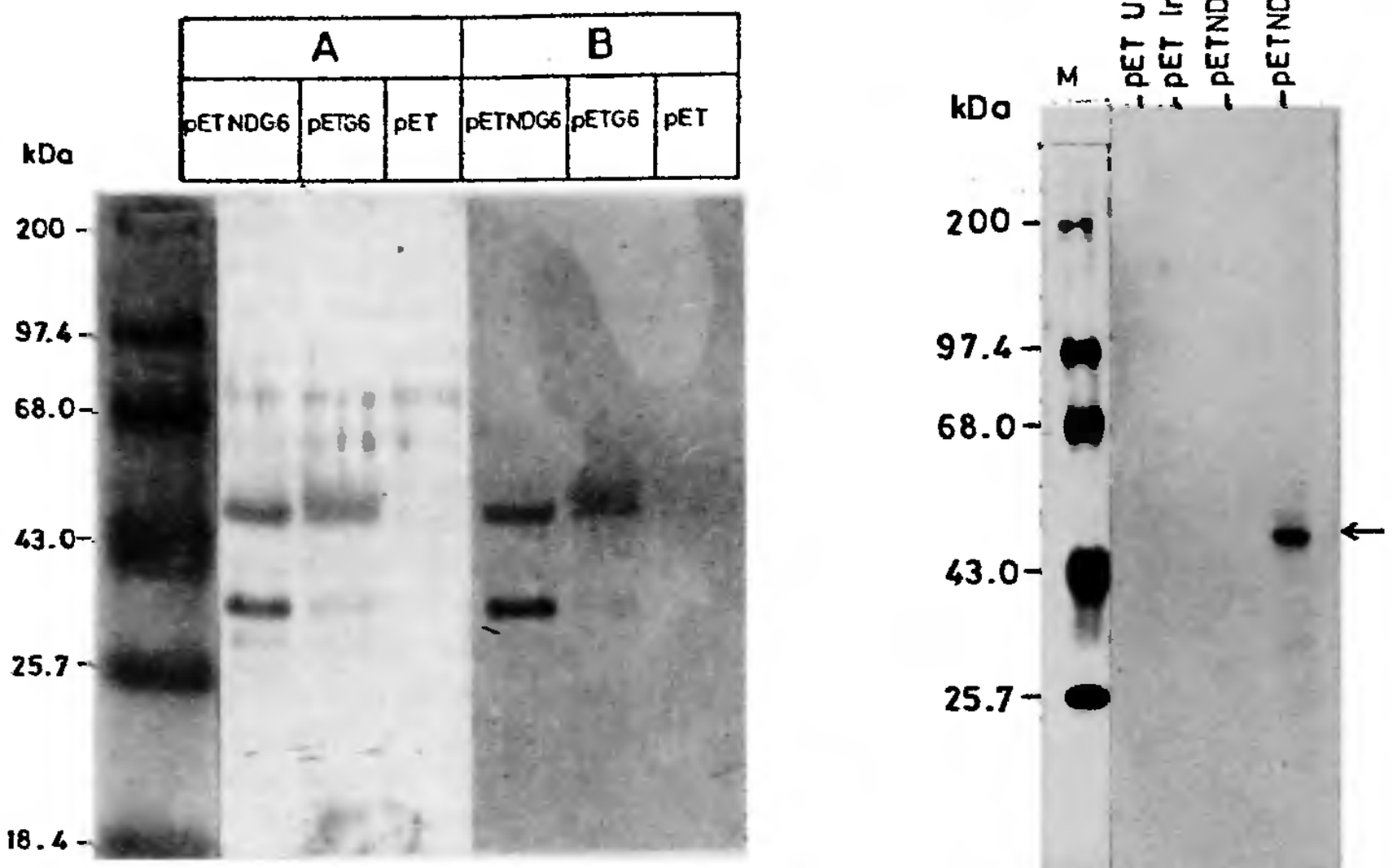

Figure 4. Immunological analysis of VP6 expressed in *E. coli*. Total bacterial cell lysates in Laemmli buffer containing β-ME were electrophoresed on a 12% SDS-polyacrylamide gel. In panel A, the proteins were directly blotted onto a nitrocellulose membrane. In panel B, the proteins were allowed to renature by soaking the gel in renaturation buffer as described in materials and methods. The recombinant VP6 in panel A was detected using polyclonal antiserum raised against the gel-eluted VP6 and in panel B, the SGI-specific mAb 255/60 was used for VP6 detection. Note that the polyclonal antiserum cross reacts with some bacterial proteins migrating above the VP6 band. The small molecular weight 34 kDa protein is also recognized by both polyclonal and monoclonal antibodies. In panel C, the radioactively labelled protein was immunoprecipitated with the SGI-specific mAb 255/60. Un, uninduced, In, induced.

UK strain and exhibited 94.48 and 98.74 per cent nt and aa sequence identities, respectively. Interestingly, IS2 VP6 shared significantly less nt sequence identity (86.74%) with the SGI VP6 from another human strain S2, though the per cent aa sequence identity was 97.23 (Table 1). These observations suggested that the VP6 gene in the Indian IS2 strain of human rotavirus most likely originated from a bovine rotavirus through genetic reassortment in nature. This is not surprising because in earlier studies in our laboratory we have isolated a large number of G10P11 type strains, from asymptomatic neonates in Bangalore and Mysore[18,41], that are reassortants between a G10P11 bovine rotavirus and a human rotavirus[9,18,42]. Recently we have also reported isolation of strains having 'long' RNA pattern and SGI specificity from children with diarrhoea that are likely to have animal origin but distinct from the G10P11 type asymptomatic strains[41]. A G9P11 type asymptomatic reassortant rotavirus has also been reported from New Delhi[43]. Thus it is possible that IS2 and the related serotype G2 and G2-like strains circulating in India[41] are also reassortants in which the VP6 gene is derived from a bovine rotavirus.

Substitutions by lysine at aa positions 97 and 134 in IS2 VP6 are interesting in the context of the reported substitution by acidic amino acids at several positions in IS2 VP4 (ref. 22). These complementary mutations in IS2 VP6 and VP4 might be of evolutionary significance in stabilizing the interaction between these two proteins in the virions of G2/G2-like Indian strains. Moreover, the presence of clusters of conserved basic amino acids in VP6 from aa position 100 to 154 suggests a functional role for this region.

Though the SGI VP6 from the simian virus SA11 was expressed in *E. coli* as a fusion protein, in insect cells as well as by recombinant vaccinia virus in MA104 cells, expression of VP6 from a human rotavirus has not yet been reported. Using the pET expression system, we have expressed the IS2 VP6 in nonfusion form at high levels and purified by single-step affinity chromatogra-

phy using Ni^{2+}-NTA-agarose as the matrix. The observation that the recombinant VP6 expressed in *E. coli* formed inclusion bodies as soon as it was formed is not surprising from the fact that majority of the VP6 expressed in insect cells was also found to form insoluble complexes[40,44]. One of the reasons for the accumulation of VP6 expressed from pETG6 in the unprocessed form could be the nonavailability of the protein for processing due to aggregation as soon as it was synthesized. Alternatively the system is limited to process large amounts of the recombinant protein.

Native VP6 exists as trimer[38]. There were conflicting reports on the nature of interactions between the monomers in the trimeric form of VP6. Gorziglia *et al.*[38] reported involvement of hydrophobic and charge interactions between the monomers in the VP6 trimer in the virion and disulphide bonds in intramolecular organization and hexamer formation. But Estes *et al.*[20] reported existence of disulphide bonds between the monomers in the trimer formed from the VP6 expressed in insect cells which was similar to the native VP6 in biochemical and immunological properties. In the present study, VP6 expressed in *E. coli* was found to be capable of forming oligomeric structures (dimers and trimers) and to possess both inter and intramolecular disulphide bridges as observed by Estes *et al.*[20].

Although VP6 represents the intermediate capsid protein, it is the major antigen of rotavirus and infected individuals have high-levels of antibodies against VP6. This is probably due to the fragility of the outer capsid resulting in the exposure of VP6 as well as the high abundance of VP6 in the virion compared to other viral proteins. Thus VP6 represents ideal candidate antigen for detection of rotaviruses in clinical samples. Coexpression of VP6 with VP2 or VP7 in the presence or absence of VP4 in insect cells has been shown to result in the formation of double-layered and triple-layered virus-like particles (VLPs), respectively, which are similar to the native particles and have been proposed as candidates for vaccine development[45]. VP6 being an excellent immunological carrier[16], availability of VP6 in large quantities should facilitate its use in vaccines as well as studies on the structure and mapping of the domains of interaction with the spike protein VP4.

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Undernutrition and aging: Effects on DNA repair in human peripheral lymphocytes

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Subjects of Indian population belonging to 3 age groups – young (8–14 yrs), adult (20–35 yrs) and old (≥ 55 yrs) were divided into 'normal' and 'undernourished' groups based on Body Mass Index (BMI) and history of diet consumption. DNA repair markers like unscheduled DNA synthesis (UDS), activities of DNA polymerase β and two endodeoxyribonucleases, (UV- and AP-DNases) were studied in the lymphocytes of these subjects under different conditions. The 'undernourished' group showed higher activities of these enzymes and also a reduced decline in age-related DNA repair capacity. These results provide evidence for beneficial effects of reduced calorie consumption in humans as well.

THE integrity of DNA, maintained by a number of DNA repair systems, is essential for the survival of cells and organisms[1,2]. Numerous physical and chemical factors damage DNA *in vivo* with their origin being both endogenous and exogenous. The resultant DNA damage has been associated with various biological end points, including cancer, mutation, birth defects, aging and other age-associated diseases[3]. A positive correlation has been claimed between maximum life span and the capacity to repair UV induced DNA-damage, both across species[4,5], as well as within and among closely related species[6–9].

In a different direction, dietary restriction (DR) is the only environmental paradigm that has been demonstrated to increase maximum achievable life span in a variety of species[10–13]. DR has also been reported to modulate the rate or eliminate the occurrence of almost all age-associated degenerative diseases. Furthermore, it is well documented that DR reduces the incidence of both naturally occurring and induced tumours[14–19].

It is possible that the mechanism behind the beneficial effects of dietary restriction is positive affectation of DNA repair potential by that regime. Reports, from this laboratory as well as from elsewhere, have indicated that DR does lead to improved DNA repair capacity in experimental animals[20–22]. However, such effects are yet to be demonstrated in humans. We have therefore, taken up a study on healthy subjects (both sexes) of Indian population living in their natural conditions, with no familial history of organic defects and premature deaths, and belonging to 3 age groups, young (8–14 yrs), adult (20–35 yrs), and old (≥ 55 yrs). At each age, the subjects were divided into two groups of 20 numbers each: one group referred to as normal (NBMI) in which the individuals with a Body Mass Index (BMI)[23] of around 20 or more are included. The other group consisted of individuals with a low BMI of 18 or less (LBMI). Care is taken to see that the range is 16–18 with only a few subjects (5 out of 60) showing marginally lesser than 16.

Table 1 *a*. Average BMI of experimental subjects

	Average BMI	
Age	N	UN
Y	22.5 ± 1.3	16.5 ± 0.5
A	21.5 ± 1.8	17.1 ± 0.7
O	23.0 ± 2.2	17.0 ± 0.7

Subjects, based on their BMI, were categorized as 'normal' (BMI ≥ 20) and 'undernourished' (BMI ≤ 18). The average BMI of the subjects (20 nos) in the various age groups are shown, where N = Normal (NBMI) and UN = Undernourished (LBMI). Y – subjects grouped as 'young' (8–14 yrs); A – subjects grouped as 'adult' (20–35 yrs); O – subjects grouped as 'old' (≥ 55 yrs).