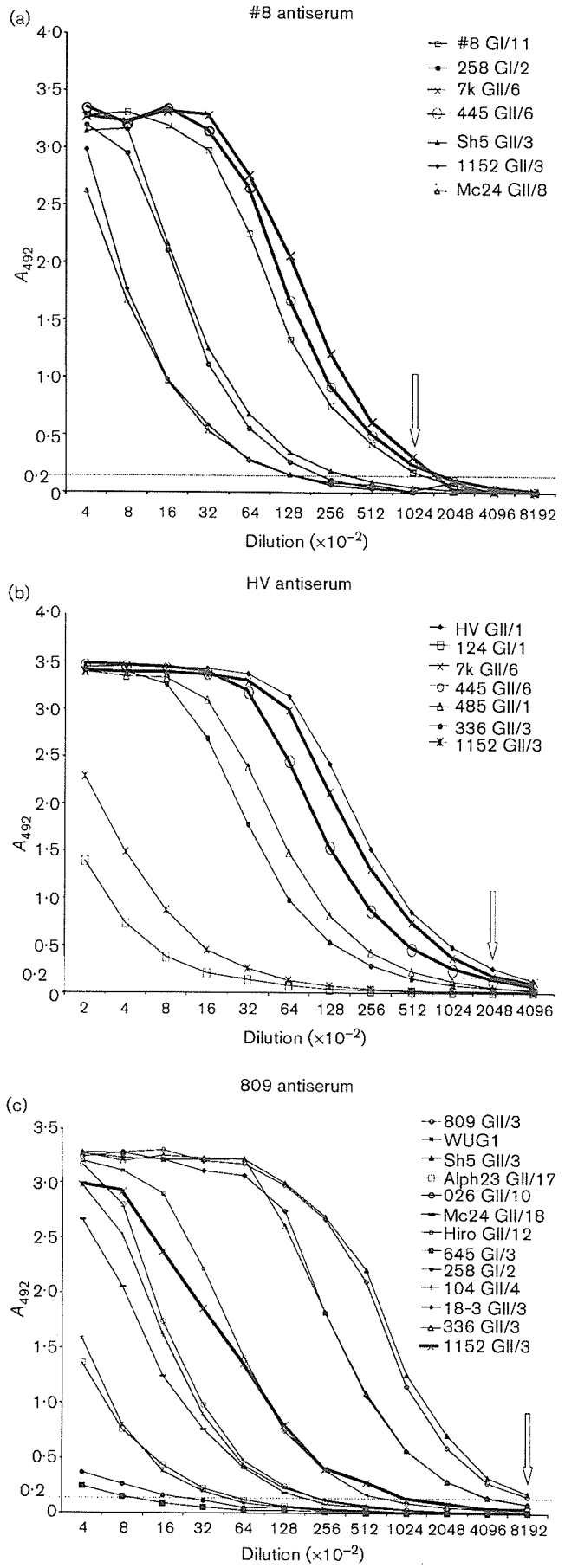
Table 2. Antibody ELISA cross-reactivity among the 26 different VLPs and antisera

Titres are expressed as $\times 10^{-2}$.

Genogroup			GI						GII																			
	Genotype	VLPs	1	2	3	4	8	11	1	1	2	3	3	3	3	3	4	5	6	6	7	8	8	10	12	12	14	17
	Antiserum	Strains	SeV	258	645	CV	WUG1	#8	HV	485	Ina	809	Sh5	18-3	336	1152	104	754	7k	445	10-25	Mc24	U25	026	CHV	Hiro	47	Alph23
GI	1	SeV	**8192**	1042	1024	1024	1024	1024	256	128	256	256	256	256	256	128	128	256	256	256	128	128	256	256	256	256	256	256
	2	258	512	**8192**	512	1024	512	512	64	16	64	32	128	64	32	16	128	128	64	64	32	32	64	128	32	64	32	128
	3	645	256	256	**8192**	256	512	1024	32	8	32	64	128	128	64	64	64	64	32	64	128	128	128	128	16	32	16	64
	4	CV	128	512	256	**8192**	512	128	64	4	32	64	128	64	32	64	32	32	32	32	64	64	64	64	16	64	32	64
	8	WUG1	256	512	256	256	**8192**	256	32	32	16	32	64	64	64	16	32	32	32	32	64	8	32	32	32	16	32	64
	11	#8	128	128	128	256	256	**1024**	256	128	256	256	256	256	256	128	256	256	1024	1024	256	128	128	256	128	256	128	256
GII	1	HV	16	16	8	16	32	256	**2048**	512	128	128	256	512	512	32	256	256	2048	1024	128	128	256	512	512	512	256	256
	1	485	128	128	16	128	128	128	1024	**4096**	128	256	256	256	256	64	128	256	128	128	128	64	256	256	512	256	256	128
	2	Ina	<4	32	16	16	64	32	256	256	**4096**	128	128	256	128	64	512	512	256	128	128	256	128	512	256	512	128	128
	3	809	<2	4	4	8	32	4	512	128	128	**8192**	8192	4096	4096	1024	64	256	64	128	256	128	256	128	256	512	64	32
	3	Sh5	8	16	16	16	16	16	128	64	64	2048	**2048**	1024	1024	256	128	128	64	64	128	64	128	128	128	128	64	64
	3	18-3	16	16	16	16	32	32	64	64	64	256	512	**1024**	512	128	64	64	64	64	128	64	128	128	128	128	64	64
	3	336	32	16	32	32	32	64	128	128	128	1024	1024	1024	**2048**	512	128	128	128	128	256	128	256	128	256	256	128	128
	3	1152	<4	<4	<4	<4	8	<4	16	16	32	512	1024	1024	1024	**1024**	32	64	32	32	32	32	32	32	16	32	8	16
	4	104	32	64	128	64	256	128	512	512	512	512	512	1024	1024	128	**8192**	1024	256	512	512	256	512	512	512	512	512	256
	5	754	128	128	64	128	256	512	1024	1024	1024	512	1024	1024	1024	256	512	**8192**	512	1024	512	256	512	1024	1024	1024	512	512
	6	7k	64	128	64	128	128	1024	512	128	128	128	256	256	128	32	256	256	**4096**	2048	128	64	256	256	256	256	128	256
	6	445	16	16	8	16	32	128	64	32	64	128	128	128	128	64	64	64	2048	**4096**	64	64	64	128	64	64	64	128
	7	10-25	32	64	128	128	64	128	512	512	256	1024	512	1024	512	128	512	512	512	512	**16384**	512	1024	512	512	512	512	256
	8	Mc24	8	16	4	8	16	NT	64	32	NT	16	NT	32	32	8	32	32	16	16	128	**2048**	2048	32	32	32	16	4
	8	U25	16	32	32	32	32	32	256	256	128	128	256	256	256	64	256	256	128	128	512	4096	**4096**	256	256	256	128	128
	10	026	32	32	32	64	128	16	512	512	256	128	128	256	256	128	256	512	256	256	512	256	256	**2048**	512	512	256	128
	12	CHV	32	64	64	64	128	128	1024	1024	512	256	256	512	64	512	256	512	256	256	256	128	512	1024	**4096**	2048	512	256
	12	Hiro	<2	16	4	2	8	4	256	128	64	64	64	64	64	16	64	128	32	64	64	32	64	128	1024	**1024**	64	32
	14	47	128	128	64	256	256	256	512	256	512	512	512	512	512	128	512	512	256	256	256	128	512	512	512	512	**4096**	256
	17	Alph23	64	64	32	32	8	32	64	32	128	32	128	64	32	256	64	64	64	64	256	64	128	256	64	256	32	**2048**



which was equal to the homologous VLP titre. We also found that GII/1 HV antiserum cross-reacted strongly (i.e. equal to the homologous VLP titre) against GII/6 7k VLPs (titre 204 800) and moderately strongly (i.e. twofold lower than the homologous VLP titre) against GII/6 445 VLPs (titre 102 400) (Fig. 3b and Table 2). We observed several antisera that cross-reacted moderately against different genotypes (i.e. fourfold lower than the homologous VLP titres). For example, GI/11 #8 antiserum cross-reacted moderately with GI/4, GI/8, GII/1, GII/2, GII/3, GII/4, GII/5, GII/7, GII/10, GII/12 and GII/17 VLPs (Fig. 3a and Table 2). GII/1 HV antiserum also cross-reacted moderately with several different genotypes, including GII/1 (strain 485), GII/3, GII/10 and GII/12 (Fig. 3b and Table 2). GII/1 485 antiserum cross-reacted moderately only with GII/1 HV VLPs; GII/6 7k antiserum cross-reacted moderately with GI/11 VLPs; GII/10 026 antiserum cross-reacted moderately with several different genotypes, including GII/1, GII/5, GII/7 and GII/12; and GII/12 CHV antiserum cross-reacted moderately with GII/1 and GII/10 VLPs (Table 2).

Genotype-specific reactivities

We observed weak cross-reactivities among different genotypes (i.e. greater than eightfold dilutions). We found that GI/1, GI/2, GI/3, GI/4 and GI/8 antisera cross-reacted weakly with other genotypes (Table 2). We also observed similar weak cross-reactivities with GII/1 (strain 485), GII/2, GII/3 (all five strains), GII/4, GII/5, GII/6 (strain 445), GII/7, GII/8 (both strains), GII/14 and GII/17 antisera. For several GII genotypes, only one type of antiserum was produced, but for five GII genotypes, we produced two or more different antisera against VLPs belonging to the same genotype (Table 2). Some interesting results were observed. For example, the antigenicities of HV and 485 were considerably different, despite the fact that both strains belong to GII/1 and share approximately 94 % amino acid identity. As shown in Fig. 3(b), HV antiserum cross-reacted strongly with GII/6 VLPs, but 485 antiserum showed little cross-reactivity with these GII/6 VLPs (Table 2). This unusual cross-reactivity pattern was also observed with other antisera. For example, for GII/6, we found that 7k antiserum cross-reacted moderately with GI/11 #8 VLPs, whereas 445 antiserum cross-reacted weakly (i.e. 32-fold lower than the homologous VLP titre; Table 2). More uniquely, we found that GII/3 1152 antiserum, which was genotype-specific, had unusual antigenicity. We found that

Fig. 3. Antibody ELISAs for NoV VLPs. Wells were coated with 100 µl purified VLPs. After washing, hyperimmune rabbit antiserum raised against the VLPs was used to detect antigens. Antisera were diluted twofold in PBS-T-SM from a starting dilution as indicated (dilutions × 10^{-2}). The arrows indicate the endpoint. (a) GI/11 #8 antiserum cross-reacts strongly with GII/6 7k and 445 VLPs. (b) GII/1 HV antiserum cross-reacts strongly with GII/6 7k and moderately strongly with 445 VLPs. (c) GII/3 809 antiserum cross-reacts weakly with GII/3 1152 VLPs.

three different GII/3 antisera (strains 809, Sh5 and 18-3) cross-reacted weakly with 1152 VLPs (i.e. eightfold lower than the homologous VLP titre; Table 2 and Fig. 3c). This unusual cross-reactivity result was not evident with the other genotypes in which we produced two different antisera (i.e. GII/1, GII/6, GII/8 and GII/12; see Table 2).

Amino acid alignment and secondary structure prediction

An alignment of 25 VP1 amino acid sequences used in this study (Mc24 complete capsid was unavailable) revealed that the N-terminal region (aa 1–49), shell domain (aa 50–225) and P1-1 domain (aa 226–278) had more conserved short continuous residues than the P2 domain (aa 279–405), P1-2 domain (aa 406–520) and C-terminal region (Fig. 4). These continuous residues may be the reason for the cross-reactivity among different genotypes, in particular, the strong cross-reactivity of #8 antiserum against GII/6 VLPs (Fig. 3a). However, this does not explain why GII/3 1152 VLPs cross-reacted weakly with GII/3 809, Sh5 and 18-3 antisera (i.e. eightfold lower than the homologous VLP titre) and moderately against GII/3 336 antiserum (i.e. fourfold lower than the homologous VLP titre). An amino acid

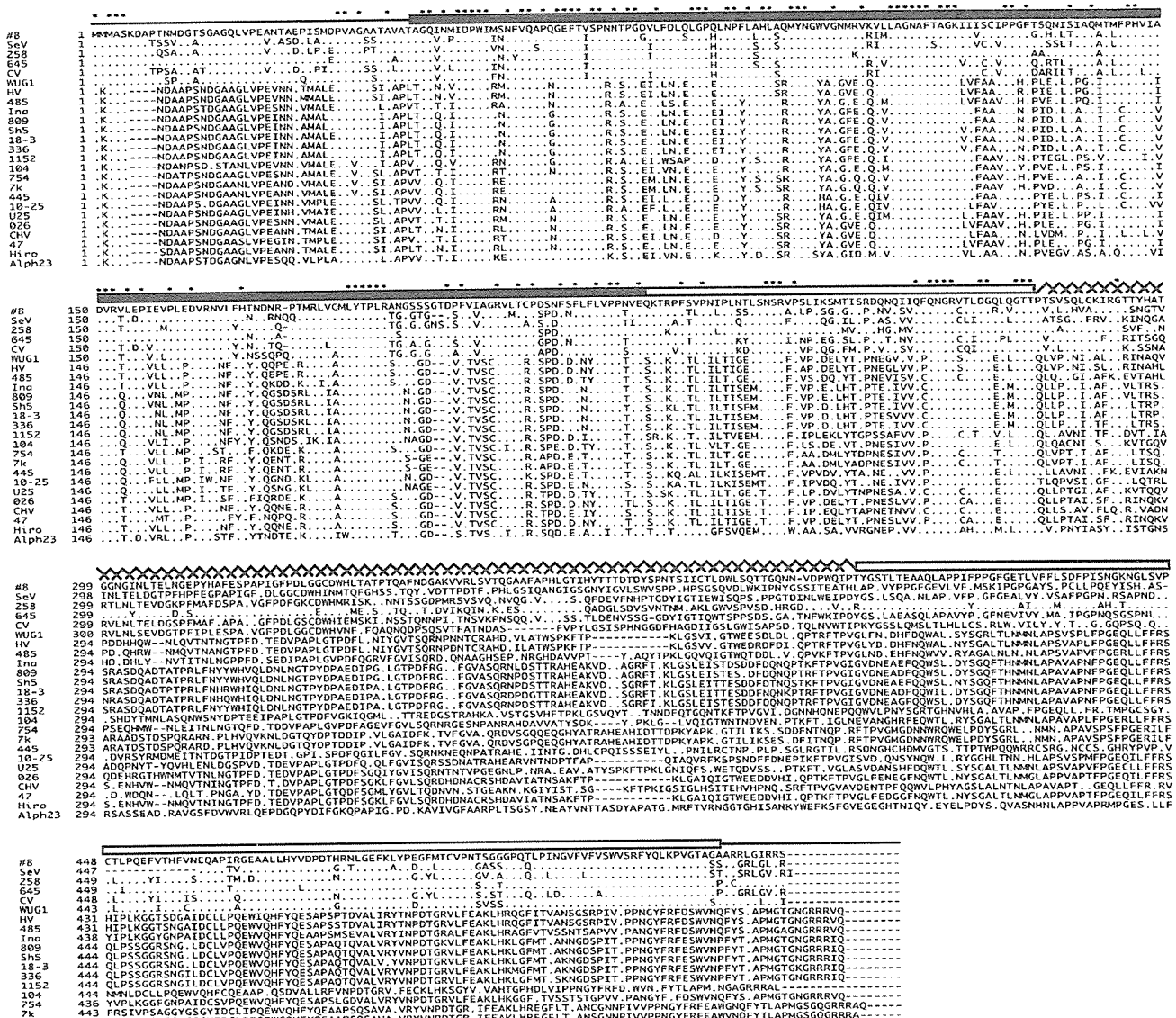


Fig. 4. Amino acid alignment of VP1 sequences of the NoV sequences examined in this study. The following regions are indicated above the sequences (in order): N-terminal region (line); shell domain (filled box), P1-1 domain (open box); P2 domain (XXX); P1-2 domain (open box) and C-terminal region (line) (Chen *et al.*, 2004). Asterisks indicate conserved amino acids.

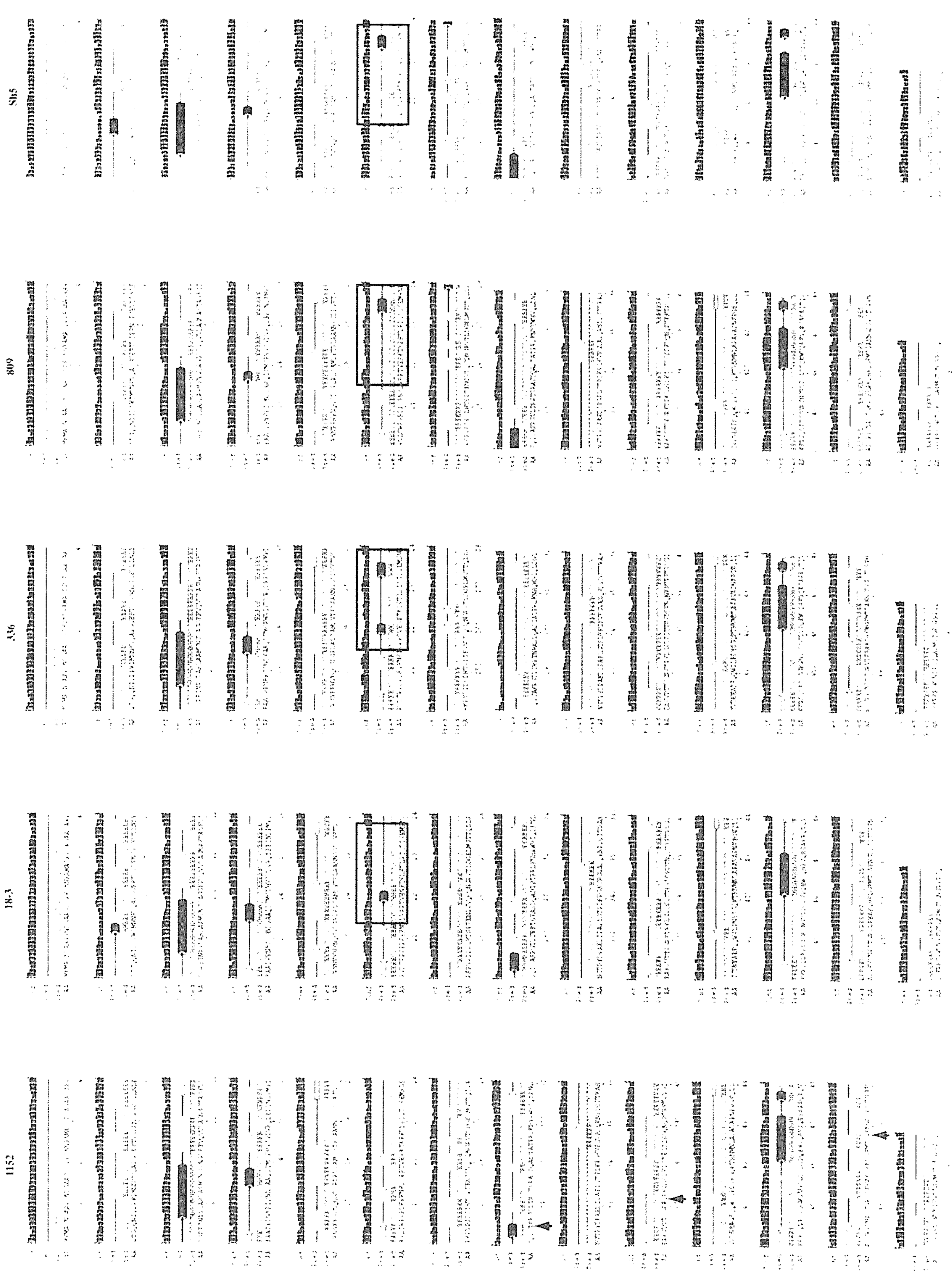

Sh5
809
336
18-3
1152

Table 3. Summary of cross-reactivities among VLPs

Each letter represents one strain. For example, GII/1 antiserum cross-reacted with two GII/6 strains (A and B), where A, strongly (i.e. identical to the homologous VLP titre), B, moderately strongly (i.e. twofold lower than the homologous VLP titre), and C, moderately (i.e. fourfold lower than the homologous VLP titre). For simplicity, we have excluded the homologous reactivities.

	Genogroup		VLPs												
			GI			GII									
		Genotype	4	8	11	1	2	3	4	5	6	7	10	12	17
Antiserum	GI	11 (strain #8)	C	C		C	C	CCCC	C	C	AA	C	C	C	C
	GII	1 (strain HV)						CC			AB		C	CC	
		6 (strain 7k)			C										
		10 (strain 026)				CC				C		C		CC	
		12 (strain CHV)				CC							C		

alignment of these five GII/3 VP1 sequences showed no unusual insertions, deletions or recombination sites; in fact, the shell domain was highly conserved among the GII/3 sequences (data not shown). However, the 1152 VP1 sequence had three unique amino acid residues (Thr-285, Ile-372 and Ser-508) when compared with the other four GII/3 VP1 sequences. The first two residues were located in the outermost region of the P2 domain, whilst the third residue was located within the P1 domain (data not shown). We used the PSIPRED secondary structure prediction software (McGuffin *et al.*, 2000) to compare the five GII/3 VP1 structures. We found that the predicted VP1 structures for 809, Sh5, 18-3 and 336 had a helix between residues 219 and 237, whereas this helix structure was absent for 1152 (Fig 5). These data suggested that the helix structure may play an important role in influencing the cross-reactivity among the GII/3 VLPs and antisera.

DISCUSSION

In this study, we analysed NoV capsid-based grouping and cross-reactivity among 26 different VLPs belonging to six GI and 12 GII genotypes. Using an antibody ELISA, we found that the antisera reacted strongly against the homologous VLPs with titres ranging from 102 400 to 1 638 400. As summarized in Table 3, we also observed strong, moderately strong and moderate cross-reactivities among different genotypes (i.e. equal to the homologous VLP titre and to twofold and fourfold dilutions, respectively). For example, GI/11 antiserum had a broad range of cross-reactivities, detecting two GI genotypes (GI/4 and GI/8) and 10 GII genotypes (GII/1–7, GII/10, GII/12 and GII/17); GII/1 antiserum (strain HV) had a broad range of cross-reactivities, detecting four GII genotypes (GII/3, GII/6, GII/10 and GII/12); GII/10 antisera also had a broad range of cross-reactivities, detecting four GII genotypes (GII/1, GII/5, GII/7 and GII/12); GII/6 antiserum detected GI/11 VLPs; and GII/12 antiserum (strain CHV) detected GII/1 and GII/10 VLPs.

Although antigen ELISAs are generally broadly reactive (Jiang *et al.*, 2000), this is the first report of a GI (strain #8) polyclonal antiserum cross-reacting strongly with other GII genotypes and the first report of a GII (strain HV) polyclonal antiserum cross-reacting strongly with other GII genotypes (Jiang *et al.*, 2002; Kamata *et al.*, 2005; Kitamoto *et al.*, 2002). These broad-range cross-reactivities may be due to unfolded VLPs on the microtitre plates at the high pH used (carbonate/bicarbonate buffer, pH 9·6) (White *et al.*, 1997). However, we have not found such broad-range cross-reactivities in any of our other studies (Kamata *et al.*, 2005). Conserved continuous residues in the shell and/or P1-1 domains may be the reason for these cross-reactivities against different genotypes (Fig. 4 and Table 2). However, we found that several antisera were genotype-specific, indicating that VLPs have unique epitopes.

Interestingly, we found that four types of GII/3 antisera (strains 809, Sh5, 18-3 and 336) cross-reacted moderately to weakly against GII/3 1152 VLPs (i.e. up to eightfold lower than the homologous VLP titre; Table 2). Amino acid alignments of these five GII/3 sequences revealed that 1152

Fig. 5. Schematic representations of the complete predicted secondary structures of VP1 of NoV (GII/3) strains 1152, 18-3, 336, 809 and Sh5. The level of confidence of prediction (Conf) is shown on the first line, where a tall box represents a high confidence of prediction and a short box represents a low confidence of prediction. The predicted secondary structure (Pred) is shown on the second line, where a helix is represented by a cylinder, a β-strand by an arrow and a coil by a line. The third line also shows the predicted secondary structure (Pred), where H represents a helix, E a β-strand and C a coil. The amino acid sequence (AA) is shown on the bottom line. The boxed regions in 18-3, 336, 809 and Sh5 VP1 indicate a helix structure that is absent in 1152 VP1. The amino acid residues that are unique to the 1152 sequence when compared with the other four GII/3 sequences are indicated by arrows.

had three unique amino acid residues compared with the other four GII/3 sequences (Thr-285, Ile-372 and Ser-508), two of which were located within the P2 domain (Thr-285 and Ile-372). Amino acid secondary structure predictions made using the PSIPRED secondary structural prediction software revealed that the VP1 secondary structures for 809, Sh5, 18-3 and 336 had a helix structure between residues 219 and 237; this helix structure was absent for 1152 (Fig. 5). This helix structure may, in part, influence the cross-reactivity among the GII/3 VLPs (i.e. without the helix structure); GII/3 1152 VLPs cross-reacted weakly with the other four GII/3 antisera. This suggestion may also explain NoV virulence in which some strains appear to infect a certain population over an extended period of time (Dingle, 2004; Noel *et al.*, 1999). In a recent report, single amino acid changes were suggested to represent a possible way for the virus to evade the host immunity (Dingle, 2004). In addition, one report suggested that a change in VP1 secondary structure (i.e. the disappearance of a helix structure) was responsible for a chronic NoV infection in an immunocompromised patient for over 2 years (Nilsson *et al.*, 2003).

Almost half of our constructs (strains SeV, 645, CV, HV, Ina, 809, Sh5, 18-3, 1152, 104, 754, CHV and Alph23) did not include the ORF3 sequence, which encodes a minor capsid protein (VP2) thought to increase the stability of NoV VLPs and may function in RNA genome packaging (Bertolotti-Ciarlet *et al.*, 2003). For rabbit haemorrhagic disease virus, VP2 is essential for the production of infectious virus (Sosnovtsev & Green, 2000). Nevertheless, we found that all constructs with or without ORF3 sequences expressed VLPs that were morphologically similar to native NoV (Fig. 2). Further studies are needed to determine whether VP2 has some influence on antigenicity.

In conclusion, this cross-reactivity study represents the most extensive undertaken for any genera in the family *Caliciviridae*. Since human NoV strains cannot be propagated in cell culture systems and human serological studies have found that VLPs and native virions share similar antigenic properties, VLPs have been used to understand antigenic relationships in more detail. Further studies, such as high-resolution structural analysis of other NoV genotypes and antigenic mapping, are needed in order to explain the complex NoV antigenicity, as previously suggested (Chen *et al.*, 2004). Finally, the results and reagents from this study can be used to design detection systems capable of detecting a broad-range of genotypes in clinical specimens; in particular, GI/11 antisera may be capable of detecting at least 32 % (12/37) of the recently described NoV genotypes (Kageyama *et al.*, 2004).

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Editor-Communicated Paper

High Efficiency Cross-Reactive Monoclonal Antibody Production by Oral Immunization with Recombinant Norwalk Virus-Like Particles

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***Abstract*: Monoclonal antibodies (MAbs) are important for in-depth antigenic characterization and diagnosis of infections with human caliciviruses that cause almost all outbreaks of nonbacterial gastroenteritis. We compared different routes of immunization with nonreplicating virus-like particles (VLPs) from recombinant Norwalk virus (rNV) and recombinant Mexico virus (rMX) administered to BALB/c mice to determine the efficiency of hybridoma production. Oral immunization with VLPs without adjuvant resulted in high yields of MAb-secreting hybridomas (90%) to these VLPs of IgG (61%), IgM (29%) and IgA (10%) isotypes. Fusions with mesenteric lymph node lymphocytes yielded MAbs of various subclasses including IgG2a, IgG3, IgM and IgA. These results suggest that an immunization route that mimics the natural route of viral infection pathway may facilitate MAb technology by increasing the yields of antibody secreting hybridoma cells.**

***Key words*: Monoclonal antibodies, Oral immunization, Virus-like particles (VLPs), Norovirus**

Caliciviruses that infect humans include Norwalk virus (NV), Mexico virus (MX) and other noroviruses (NoVs) that are the most common cause of viral gastroenteritis in young children and adults worldwide (4, 22, 28). Although a murine norovirus that is cultivatable has recently been identified (29), the human viruses cannot be cultivated in cell culture and no animal model is available (7). In spite of these difficulties, the genome organization and biological and biochemical properties of these viruses have been characterized using virus from stool samples obtained from volunteer studies (7, 8, 16).

Cloning of the NV genome led to the complete sequence that showed that NV contains a single-stranded RNA genome of positive polarity; the virus capsid shell is composed of 180 molecules of a single major protein (VP1) with an apparent molecular weight of 58,000 daltons (58K) and a few molecules of a second protein called VP2 (5, 13, 15, 16, 23). Baculovirus expression of the NV capsid gene, produced empty, recombinant Norwalk virus-like particles lacking the viral genome (rNV VLPs) (15). Because the VLPs are structurally and antigenically similar to the native NV particles, hyperimmune antisera produced in animals to these VLPs were expected to be a milestone for the diagnosis of NV and NV-related gastroenteritis. Enzyme-linked immunosorbent assays (ELISA) to detect antigen in stool samples or serologic responses of patients have been developed (6, 8). However, while these assays are sensitive and easy to perform, the antisera to rNV VLPs exhibit a restricted reactivity and any specific polyclonal antiserum only detects a subset of closely related noroviruses (17).

MAbs are useful tools for the detection and characterization of viral proteins. However, the yields of hybridomas obtained following standard fusion proto-

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; ip, intraperitoneal; rMX, recombinant Mexico virus; rNV, recombinant Norwalk virus; sc, subcutaneous; VLPs, virus-like particles.

cols are not predictable. Procedures, such as the routes of immunization for the mouse (12, 20), the types of fusion partner (25) and the culture conditions such as media supplements (26) continue to be modified to improve the efficiency of hybridoma production.

Previously, we developed MAbs (11) that react with recombinant Norwalk virus (rNV) (15) and recombinant Mexico virus (rMX) (14) particles by using the common intraperitoneal immunization route. Although the resulting MAbs were useful to characterize the native virus, they only recognized closely-related Norwalk like virus (NLV) strains (11), and the yields of MAbs were low. NV is naturally spread by the fecal-oral route and sera from adults cross-react by ELISA with many NoVs in the *Caliciviridae* family (27), and rNV VLPs have been shown to induce mucosal and systemic antibody responses when delivered orally to mice or volunteers without adjuvant (2, 3, 9). This paper reports evaluation of the effect of oral immunization on the yield of MAb-producing cells and isotypes and subclasses of MAbs produced.

Materials and Methods

Virus-like particles (VLPs). VLPs of rNV and rMX were produced by using the baculovirus expression system as described previously (14, 15).

Immunization of BALB/c mice with VLPs. Six week old BALB/c female mice were immunized using protocols that received institutional approval and followed two experimental designs.

Experiment I: Recombinant NV or rMX VLPs [10 μg in sterile phosphate buffered saline (PBS), pH 7.2], were used to immunize two or three mice, four times at weekly intervals by either a) intraperitoneal injection (ip) or subcutaneous injection (sc) with Freund's complete adjuvant for the first injection and without adjuvant for the subsequent injections, or b) by oral administration without adjuvant. After the fourth immunization, mice immunized by either route were boosted by an intravenous (iv) injection of the same dose of VLPs without adjuvant into the tail vein 3 or 4 days before cell fusion. The mice were kept on a normal feeding schedule and diet. The yields of hybridoma cells were compared among mice based on the different routes of immunization.

Experiment II: Two groups of mice, each containing two mice, were immunized orally. Group 1 mice were administered 5 μg of rNV in sterilized PBS, pH 7.2, orally without adjuvant daily for 4 consecutive days. A boost of 5 μg of rNV VLPs was administered into the tail vein on the fifth day and the fusion was performed 3 days later. Group 2 mice were immunized orally with the same dose of rNV without adjuvant on days 1, 3, 4 and 6 followed by the same booster intravenous injection on day 7. Fusions were performed 3 days later. In order to enhance the uptake of rNV VLPs in PBS, the mice in these two groups were kept without water overnight prior to the oral inoculations early the next morning. After immunization, water was supplied normally.

Myeloma cells and fusion. Myeloma cells P3 (P3X63Ag8U.1) and PAI (kindly provided by Dr. Kotani, Tokyo Metropolitan Institute of Medical Science, Tokyo) were used for fusion. P3 or PAI myeloma cells were cultured in Dulbecco's Modified Eagles' Medium (DMEM) in the presence of 15% fetal calf serum (FCS). In experiment I, splenocytes($1\ 2\times10^{7}$ cells/ml) were fused with P3 or PAI cells at a ratio of 2:1 as described previously (11). After cloning in medium supplemented with hypoxanthine-aminopterin-thymidine, the cells were cultured with DMEM containing 15% FCS. Culture supernatants were screened by ELISA using plates coated with VLPs as described below.

In experiment II, mesenteric lymph nodes lymphocytes were harvested and the cells from two mice in each group were pooled prior to fusion with PAI myeloma cells. Pooled splenocytes from two mice were also fused with the PAI myeloma cells. The number of splenocytes was adjusted to be the same as that of the mesenteric lymphocytes because the number of lymphocytes in the mesenteric lymph nodes was less than that from the spleen. All experimental parameters from cell fusion to MAb screening assay were kept constant between experiments I and II.

Enzyme-linked immunosorbent assay (ELISA) for screening MAbs. ELISA test was performed following the protocol previously described (11) with slight modifications. Briefly, 96-well plates (Sumitomo Bakelite Co., Ltd.) were coated with 100 ng per well of rNV or rMX VLPs in 50 μl of 0.01 M PBS, pH 7.2, for 4 hr at room temperature. Following a 2-hr reaction with each test hybridoma supernatant at 37 C and further washing with PBS containing 0.05% Tween 20 (PBS-T), horseradish-peroxidase labeled anti-mouse IgG, IgM and IgA (Cappell Laboratories, Westchester, Pa., U.S.A.) were reacted separately for each clone for 2 hr at 37 C. After a final wash with PBS-T, 0.1 M ABTS [2,2 azinobis(3-ethylbenzothiazoline-6-sulfonic acid in 0.1 M, pH 4.0 citrate-phosphase buffer)] was added as the substrate in the presence of H_2O_2. After a 20-min reaction, the optical density (OD) at 405 nm and 630 nm were measured with a Microplate Reader (Bio-Rad., Hercules, Calif., U.S.A.).

Cross-reactive assay by ELISA with MAbs and

genogroup I and genogroup II VLPs. In order to confirm cross-reactivity of the MAbs, ELISA assays were performed as previously described (11). Briefly, both genogroup I and genogroup II VLPs including rNV, Seto 124 (r124), Chiba 407 (rCV) and Funabashi 258 (r258) for genogroup I, Snow Mountain agent (rSMA), Glimsby (rGV), rMX, Kashiwa 47 (r47), Narita 104 (r104), Chitta 76 (r76) and Ueno 7K (r7K) for genogroup II and rSapovirus were coated using the same procedures as above. rCV, r124, r258, r47, r104, r76 and r7K were kindly supplied by Dr. Natori and Dr. Takeda, NIID, Tokyo. Cross-reactivity by ELISA was determined to occur if the optical density (OD) ratio between the test sample and negative control (PBS) was more than 20.

Evaluation of hybridoma production. All supernatants in the hybridoma colonies were harvested and assayed by ELISA. The percentage of hybridomas secreting VLPs-specific antibody per total hybridomas were evaluated. The calculations were performed separately by the fusion partner in experiment I or by the site of harvesting of lymphocytes used for the fusion in experiment II. The results, presented as percentages, were compared between groups, immunization route, with the Z-test of proportions. A P-value of <0.05 was considered statistically significant.

Results

Yields of MAb-Producing Hybridomas and Immunoglobulin Subclasses of MAbs

More than 90% of hybridoma cells from the orally immunized mice in experiment I produced MAbs (Table 1). In contrast, only 13 to 45% of the cells from mice immunized by the ip or sc routes produced MAbs. Production rates were similar using either the P3 or the PAI myeloma cells for fusion. The immunoglobulin classes of the MAbs obtained from mice immunized orally were IgG, IgM and IgA with 61%, 29% and 10%, respectively (Table 2). In contrast, the MAbs obtained from mice given ip immunization were IgG, IgM and IgA with 85%, 12% and 3%, respectively. Following the rapid oral immunization protocol (experiment II), yields of MAb-hybridomas were greater using the mesenteric lymph node lymphocytes than splenocytes (Table 3). For example, 80% of hybridomas came from mesenteric lymph node lymphocytes, but 20% of hybridomas were from splenocytes, in the group 1 mice harvested 5 days post oral immunization.

In the group 2 mice, the rate of MAb-producing hybridomas was 72% from mesenteric lymph node lymphocytes and 28% from splenocytes. The numbers of mesenteric lymph node lymphocytes that produced MAbs were not increased significantly; however, the yield of MAb-secreting hybridomas from the splenic lymphocytes was increased 3-fold when the lymphocytes were harvested later after the last oral immunization.

The immunoglobulin classes from experiment II are shown in Table 3. Hybridomas from the mesenteric lymph node lymphocytes of group 1 mice in experiment II mainly (75%) produced IgM although some (25%) produced IgA. Hybridomas from splenocytes produced IgM and IgA equally. Hybridomas from mesenteric lymph node lymphocytes from the group 2 mice produced IgM (61%), IgA (22%), IgG2a (6%) and IgG3 (11%), but hybridomas from splenocytes produced IgM (14%), IgA (14%) and IgG1 (71%).

Cross-Reactivity of the Hybridomas Differs Depending on Route of Immunization

Cross-reactivities of the MAbs with a variety of VLPs were tested by using VLPs-coated ELISAs as described previously (11). A single dose of rNV VLPs used for oral immunization produced broadly cross-reactive MAbs not only against genogroup I but also

Table 1. Yields of hybridomas producing MAbs to rNV and rMX following intraperitoneal (ip), subcutaneous (sc) or oral immunization (oral)

Immunization route	Virus immunogen	Myeloma	MAb-producing cells/Fusion cells (%)[c]
ip	rNV	P3	31/158 (20)
ip or sc	rNV	PAI	33/74 (45), 37/296 (13)[a]
ip	rMX	PAI	9/57 (16), 42/296 (14), 37/210 (18)[b]
oral	rNV	P3	185/200 (93)
oral	rNV	PAI	179/192 (93)

ip or sc: intraperitoneal or subcutaneous immunization followed by a tail vein booster.
oral: oral administration followed by a tail vein booster.
[a] Results from two individual mice used for fusion.
[b] Results from three individual mice used for fusion.
[c] MAbs-producing cells from all ip (17%) and all oral (92%) immunization routes were significantly different ($P<0.0001$).

Table 2. Immunoglobulin classes of MAbs produced by oral or intraperitoneal (ip) immunization

Route of immunization[a]	No. of MAb-secreting hybridomas	Isotype of MAb produced (% of hybridomas)[a]		
		IgG	IgM	IgA
oral	157	96 (61)	45 (29)	16 (10)
ip	121	103 (85)	14 (12)	4 (3)

[a] Comparisons of hybridomas obtained from oral versus ip routes of immunization for all significant ($P<0.001$ for IgG; $P<0.001$ for IgM, $P<0.023$ for IgA).

Table 3. Immunoglobulin isotype and subclasses of MAbs obtained from fusions between PAI cells and mesenteric lymph node lymphocytes or splenocytes after oral immunization

	Ig isotype and subclasses from hybridomas from fusions with lymphocytes from:			
	Mesenteric lymph nodes (%) Numbers of hybridomas (n)		Spleen (%) Numbers of hybridomas (n)	
Group 1	IgG (0) IgM (75) IgA (25)	$n=8$	IgG (0) IgM (50) IgA (50)	$n=2$
Group 2	IgG2a (6) IgG3 (11) IgM (61) IgA (22)	$n=18$	IgG1 (71) IgM (14) IgA (14)	$n=7$

Lymphocytes from group 1 and group 2 were collected 5 and 7 days after oral immunization, respectively, with the same total 20 μg doses of VLPs. Cell fusions were performed 3 days later since 5 μg VLPs were boosted via a tail vein.

genogroup II VLPs (Table 4). In contrast the production of MAbs which reacted broadly were lower following ip immunization with one or mixed (rNV and rMX) doses of VLPs compared to oral immunization.

No MAbs clones were obtained to react with recombinant Sapovirus.

Discussion

The development of MAb technology (19) has contributed enormously to the field of virology and infectious diseases for biochemical investigations of viral protein functions and for new methods of diagnosis such as ELISAs. Such diagnostic procedures are simple, have high specificity, can assay many clinical samples at one time and are more economical than polymerase chain reaction (PCR) which is used predominantly today for detection of noroviruses because broadly reactive MAbs to detect these viruses remain to be produced. To isolate and characterize new MAbs, many technical modifications such as fusion procedures, fusion partners and medium supplements can be improved to obtain highly efficient yields of MAbs (25, 26).

Immunization routes are one of the important factors for efficient establishment of hybridomas that secrete specific antibodies (12, 20). The most common routes for immunizing mice for fusion are ip, sc, intramuscularly (im) and food-pad inoculations. Previously, using the common ip route, we primed BALB/c mice with VLPs and obtained MAbs, which were sufficient to characterize the rNV by Western blotting and immunoprecipitation (11). These MAbs reacted with a subset of epitopes of native NV or rNV, but they only reacted with viruses closely related to the NV immunogen (10,

Table 4. Cross-reactivity of MAbs producted by different routes following immunization with rNV and/or rMX

			G I				G II							
route	VLPs	# of MAbs clones	rNV	r124	rCV	r258	rSMA	rGM	rMX	r47	r104	r76	7K	rSV
oral	rNV	5												
oral	rNV	3												
oral	rNV	2												
ip	rNV	8												
ip	rNV	1												
ip	rNV	23												
ip	rNV	4												
ip	rNV×rMX	3												
ip	rNV×rMX	2												
ip	rNV×rMX	6												
ip	rMX	2												

OD ratio of wells containing: samples / PBS>20.

rNV and/or rMX were administered to BALB/c mouse orally (oral) or by intraperitoneal injection (ip). MAbs were tested with VLPs-coated ELISAs described in the text. MAbs were also characterized by Western-blotting (18).

11). We suspected that other cross-reactive epitope(s) exist among most or all human viruses in the *Caliciviridae* family because low titers of broadly reactive antibodies are detectable in convalescent sera from humans (27).

Human caliciviruses cause acute gastroenteritis, which is most frequently transmitted orally by contaminated drinking water or food such as fruits, salads, and shellfish including raw oysters. Ball et al. (1998) showed that oral immunization of mice with rNV in the absence of an adjuvant induces serum IgG as well as intestinal IgA antibodies. Those experiments concluded that i) the IgG response was dose-related, ii) cholera toxin (CT) used as a mucosal adjuvant influenced the magnitude of the responses, iii) intestinal IgA responses were dose-related and enhanced by immunization with CT, and iv) the kinetics of serum IgG responses were related to the day post oral administration.

To try and broaden the repertoire of MAbs to NV, we tested whether use of the oral route of immunization would change the frequency or type of hybridoma obtained. The immunogens used in the present paper were 10 μg per dose for 5 doses (50 μg/mouse) in experiment I, and 5 μg per dose for 5 doses (25 μg/mouse) in experiment II. These oral antigen doses were in the low range of the doses shown previously to induce serum and mucosal antibody responses in mice (2). However, oral immunization with these low doses resulted in a high efficiency (90%) of MAb-secreting hybridomas in the current experiments. Thus, low oral doses of rNV VLPs prime lymphocytes effectively. Although the hybridomas produced IgG as well as IgA and IgM MAbs, oral immunization produced IgA-secreting hybridomas significantly more frequently than did ip immunization. The choice of the P3 and PAI cells as the fusion partner did not significantly affect these results.

To further examine the immune response, we also compared hybridoma production using splenocytes and mesenteric lymph node lymphocytes for fusion. From these experiments, hybridomas secreting MAbs showed a coincidence with the time course in which lymphocytes migrated from the mesenteric lymph nodes to the spleen. Thus, the immunoglobulin isotypes from these hybridomas from mesenteric lymph node lymphocytes was mainly IgM, while hybridomas from splenocytes secreted variable immunoglobulin isotypes including IgM, IgG1 and IgA.

These results confirm that low doses of orally administered VLPs prime mucosal lymphocytes and these primed cells must move from the mesenteric lymph nodes to the spleen. The mechanisms regulating the shift and/or predominance of production of a specific immunoglobulin isotypes and subclass were not investigated in this study. However, the levels of cytokines such as IFN-γ, TGF-β, IL-2, IL-4 and IL-8 produced by lymphocytes that can participate in mucosal immune responses were not significantly changed when tested by PCR with specific primers (data not shown).

The MAbs produced by ip immunization had little cross-reactivity with other types of VLPs obtained from the baculovirus expression system. However, many of the MAbs obtained from mice immunized orally with one strain of VLPs cross-reacted with other strains of VLPs representing both genogroup I (rNV, r124, rCV and r258), and genogroup II (rSMA, rGV, rMX, r47, r104, r76 and r7K) Norwalk-like viruses (18), and some of these MAbs have been used to formulate an ELISA that is useful to detect noroviruses in outbreak settings (24).

Oral immunization of mice with gastroenteritis viruses may be an efficient and useful approach to obtain MAbs because it mimics the natural infection pathway, which could induce antibodies recognizing a broad range of viral epitopes. Live attenuated oral vaccines have been used to induce IgA antibody (21). Our use of oral immunization also resulted in a high rate of IgA-secreting hybridomas. IgA MAbs were produced from fusions with Peyer's patch cells (1). Our results show that the mesenteric lymph node lymphocytes that play an important role in local immunity are another source of sensitized lymphocytes for fusions before they migrate to the spleen. While splenocytes are generally used for fusions, use of lymphocytes from the local immune tissue might enhance the frequency or specificity of hybridomas that can be obtained to a variety of immunogens.

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「広域における食品由来感染症を迅速に探知するために必要な情報に関する研究」
平成 18 年度　分担研究報告書

消化管寄生性原虫類の遺伝子型解析と分子疫学的研究

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研究要旨

消化管寄生性原虫であるクリプトスポリジウムならびにジアルジアの国内感染に関して、感染および環境等の汚染を迅速に探知する必要性が高まっている。本研究では、遺伝子診断の迅速性と情報量の多さから、これを原虫類の感染実態解析に用いることが有用と考え、本年度は国内感染の原因解明が遅れているジアルジア感染に関して、疫学的な特徴を整理するとともに、ヒトならびに野生動物に関する病原体情報を解析した。

感染症発生動向調査事業年報(2000-2004 年度)の統計から、全報告数は年間およそ 100 例でほぼ一定に推移し、国内例は約 40%を占めていた。男性は女性の約 2.6 倍の報告数で、幼児—学童期での感染がほとんどないこと、35 歳前後にピーク的な集中が見られる傾向にあるが、高年齢層でも感染が見られた。国内でヒト感染した遺伝子型として Assemblage A2 ならびに B4 を検出した。ヒト特異的遺伝子型の A2 の検出は、ヒトを感染源とする伝播が存在することを示唆するものであった。一方、東北地方生息の野生ニホンカモシカより 10%前後の割合でジアルジアが検出され、遺伝子型は人畜共通感染型の Assemblage A1 と同定された。これまでの野生および飼育動物からの人畜共通感染型 A1 および B4 の検出結果と合わせ、感染源としての動物の調査の重要性が指摘された。また医療現場での下痢症病原体スクリーニングを目的とした免疫クロマト診断法について、市販キットの検出感度を調べた結果、ジアルジアに関しては蛍光抗体染色法と同等の感度が得られたが、クリプトスポリジウムに関しては判断不能で再検討が必要となった。臨床材料を用いた場合の本キットと蛍光顕微鏡の検査結果は一致しており、簡便なスクリーニング法としては有用と考えられた。

A. 研究目的

消化管寄生性原虫であるクリプトスポリジウムならびにジアルジアは、従来、旅行者下痢症、輸入下痢症の原因としてとらえられてきた。しかし近年はその国内感染が問題となりつつあり、原虫による感染および環境等の汚染を迅速に探知する必要性が高まっている。本研究では、遺伝子診断の迅速性と情報量の多さ

から、これを原虫類の感染実態解析に用いることで国内における感染源、感染経路を解明し、原虫感染症対策に資する情報を提供する。特に本年度は国内感染の原因解明が遅れているジアルジア感染に関して、疫学的な特徴を整理するとともに、ヒト臨床例に関する遺伝子診断ならびに野生動物のニホンカモシカにおける原虫類調査を行った。また、感染症の迅速な探知には医療現場で利用可能な、スクリーニングを目的とした簡易・迅速診断法が不可欠になると思われる。この点に関して、近年開発の進んでいる免疫クロマト診断法の検討も合わせて行った。

B．研究方法

B-1 国内ジアルジア感染報告に関する情報解析

国立感染症研究所、感染症情報センターが公表する感染症発生動向調査事業年報(http://idsc.nih.go.jp/idwr/CDROM/Main.html)を用いた。2000-2004 年度の統計より感染地別、性別、年齢階級別に集計された資料を用いて、国内感染例報告の集計、解析を行った。

B-2 ヒトのジアルジア症の遺伝子診断

材料:ヒトの臨床材料として HIV 陽性患者の下痢便を用いた。糞便中の原虫検査には抗クリプトスポリジウム(*C.parvum*)抗体ならびに抗ジアルジア(*G.lamblia*)抗体の FITC 標識したものを両方含む Merifluor(Mereidian 製)直接蛍光抗体を用い、糞便を酢酸エチル法で粗精製した試料を染色後、蛍光顕微鏡下で観察した。蛍光抗体法によりジアルジアシスト陽性が確認された粗精製試料を用いて SDS 煮沸法により DNA を抽出、PCR 用試料調整のためガラスパウダー法で精製した。

PCR およびシークエンス解析: GDH (Glutamate dehydrogenase)遺伝子の部分配列を PCR 法で増幅し、その増幅産物をテンプレートに Nested-PCR 用プライマーを用いてダイレクトシークエンスを行い、そのシークエンスの解析を行った。

B-3 野生ニホンカモシカの原虫類汚染実態調査

材料:岩手県および秋田県にて野生ニホンカモシカより採取した糞便をショ糖浮遊法により粗精製し、直接蛍光抗体法により *Giardia* 属ならびに *Cryptosporidium* 属原虫の検索を行った。

PCR およびシークエンス解析:16SrRNA 遺伝子の部分配列を PCR 法で増幅後、PCR 産物を用いてダイレクトシークエンスを行い、そのシークエンスの解析を行った。

B-4 免疫クロマト法の感度試験

材料:市販の Meridian 社製のクリプトスポリジウム・ジアルジア検出用免疫クロマト法キット ImmunoCard STAT を用いた。検出感度試験のためのシストは *G.lamblia* 感染スナネズミの糞便を、またオーシストは *C.parvum* (genotype2)感染ヌードマウス(BALB/c nu/nu)の糞便を用いて、ショ糖浮遊法さらに塩化セシウム法を用いてほぼ糞便挟雑物がなくなるまで精製し、蒸留水を用いて原虫濃度を 10^6/ml に調整した。また臨床試験用には、直接蛍光抗体法によりシスト/オーシスト陽性が確認された臨床材料を用いた。

試験法:試験キット添付の操作手順に従い検出を行った。また結果の判定もキット添付の判定基準に従った。

C．結　果

C-1 国内ジアルジア感染報告に関する情報解析

感染症発生動向調査事業年報の統計で、2000 年度に関しては全報告数のみを、2001-2004 年度(最新)では全報告数および感染地別集計資料を利用した。近年の動向として全報告数と国内、国外感染例の割合の年次推移を図-1 に示した。全報告数は 2000 年以後 100 例前後でほぼ一定に推移していた。国内、国外例の割合もほぼ一定しており、国内例は約 40%を占めていた。図-2 に性別、年齢階級別の国内例に限定した報告数を示した。男性 135 例(30～37 例/年)、女性 52 例(12～15 例/年)で、若年齢層では 1～4 歳で 2 例の報告があったのみで、20 歳以下ではほとんど報告がなかった。男性では最も多いのが 30～45 歳までの年齢であったが、50 歳以上でも報告数は大きくは減少しなかった。女性では年齢による差がなかった。なお全報告数に対する集計ではあるが、都道府県別では東京、大阪を中心とした大都市圏が半数以上を占める傾向にあった。

C-2 ヒトのジアルジア症の遺伝子診断

今回、国内感染が疑われる 2 例のジアルジア症例に関して、検出されたシストを用いて遺伝子診断を行った。両例ともに HIV 陽性患者で慢性下痢症を合併していた。ジアルジア感染者では、シスト陽性の場合、下痢ではなく軟～有形便であることが多いが、今回の材料の便性状は下痢性でシストが多数検出された。一例に関しては未精製の生試料よりシスト数を測定することが可能で、糞便中のシスト濃度は約 5,400/ml stool であった。これらの症例に関して GDH 遺伝子による遺伝子診断を行った結果を表-1 に示した。2 例は異なる *G.lamblia* 遺伝子型 Assembly を示し、1 例(試料 1)はヒト特異的な型として知られる Assemb A2、もう 1 例(試料 2)は人畜共通感染性の知られる Assemb B4 と同定された。国内感染例に関してこれまで検出した遺伝子型は今回の 2 種類の型に加え、別の人畜共通感染性型として知られる Assemb A1 がある。これらの遺伝子型の近縁関係を調べた結果を図-3 に示したが、系統樹からみるとヒト感染性の Assemb A1 および A2 は同じくヒト感染性の Assemb B3 および B4 よりも、ヒト以外の動物が主な宿主である Assemb E(ウシ、ブタ等家畜)あるいは F(ネコ)の方が近縁であり、*G.lamblia* の宿主特異性の複雑さを示す結果であった。

C-3 野生ニホンカモシカの原虫類汚染実態調査

今回の調査では岩手県において 65 頭、秋田県においては 33 頭の野生ニホンカモシカを検査対象とした。原虫検査の結果を表-2 示したが、*Giardia* 属のみ検出され、*Cryptosporidim* 属は非検出であった。ジアルジア検出率は岩手県の場合 6.2%(4/65 頭)、秋田県では 15.2%(5/33 頭)であり、およそ 10%前後の個体が感染していることが明らかとなった。今回の調査では感染個体の生息と水源との関連に関するデータは得られなかった。16SrRNA 遺伝子による遺伝子型別を行った結果では、検出されたシストは人畜共通感染性型として知られる Assemb A1 と同定された。

C-4 免疫クロマト法の感度試験

Meridian 社製の免疫クロマト法キット ImmunoCard STAT に関しては、他の検査方法(蛍光顕微鏡検査、ELISA)との感度比較はデータとして添付されているが、原虫の量的検出感度に関するデータがない。実際の感染を診断する上では、量的な感度のデータは重要であることから精製シスト/オーシストを用いて、10,000～100 シスト/オーシストの濃度条件で感

度試験を行った。本キットでは実際の糞便試料を調べる場合 60μl の試料を添加する。一方、蛍光顕微鏡検査1回で調べることのできる試料はおよそ 10μl で、検出感度としてはその中に含まれる数個のシスト/オーシストの存在を確認することが可能である。従ってキットにおける100シスト/オーシスト/60μlという濃度はおよそ 20 シスト/オーシスト/10μl であり、蛍光顕微鏡検査の感度であれば陽性と判断される条件である。図-4 に感度試験の結果を示したが、シストの検出感度は100で、蛍光顕微鏡とほぼ同等の検出感度が得られたが、オーシストの場合 10,000/assay でも陰性であり、その検出感度は判断不能であった。2 つのロットで試験したが、結果は同様であった。なお用いたオーシストの蛍光抗体試薬に対する反応は強陽性であった。

臨床材料を用いた試験の結果を図-5 に示した。本キットでは新鮮あるいはホルマリン固定標本が使用可能であるが、原虫の固定条件として基本的に重クロム酸カリウム溶液(5%)を用いており、固定標本に関しては同固定液で固定された試料を PBS で洗浄し、再浮遊して用いた。*Giardia* に関しては新鮮材料を検査することができた。(A)は *G.lamblia* (Assemb.A2)陽性試料、HIV 陽性患者の検査結果で、明瞭な陽性バンドが確認された。本試料は 5,400/ml のシスト濃度であることが分かっているので、検査では 6 倍希釈した試料をもちいることから、9 シスト/10μl の試料おいて陽性と判断されたことになる。一方、*Cryptosporidium* に関しては重クロム酸固定試料のみ検査した。(B)は *C.parvum* (Genotype 1) 陽性試料、HIV 陽性患者の検査結果で、試料は重クロム酸溶液で約 3 年間固定保存したものであったが所定反応時間内(15 分間)で陽性バンドが確認された。

D. 考察

現在の感染症法では、消化管寄生原虫の中の赤痢アメーバ、クリプトスポリジウムならびにジアルジアが第 5 類、全数把握対象疾患として届出の義務がある。その報告は国立感染症研究所、感染症情報センターにて集計され、感染症発生動向調査週報(IDWR)に公表される。上記原虫類のうち、赤痢アメーバとクリプトスポリジウムスポリジウムの国内感染に関しては、赤痢アメーバでは男性ホモセクシャルグループ、HIV 陽性患者、あるいは施設内感染が、クリプトスポリジウムにおいては水系集団感染が主たる要因と推定される。これに対し、ジアルジアの場合は国内感染例が 40%を占めるにも関わらず、感染源、感染経路が判然としていない。

性別、年齢階級からみた国内ジアルジア症の疫学的特徴は、諸外国では小児性下痢の主要原因となっている本症が、幼児―学童期での感染がほとんどないこと、男性は女性の約 2.6 倍の報告数であること、35 歳前後にピーク的な集中が見られる傾向にあるが、高年齢層でも感染が見られることなどがあげられる。米国 CDC の 1998-2002 年までのジアルジア症サーベランス集計結果(Hlavsa ら 2005)によれば、期間中の症例報告数はおよそ年 20,000 件で、1-9 歳と 30-39 歳の年齢層が他に比べて多いこと、季節的には夏季に発生例が多いこと、プール、公園の水施設、湖、河川での水遊びとの関連性が指摘されている。国内においても食品あるいは野外での未消毒の水の飲用、また STD を含めてのヒト-ヒト間の接触感染および動物からの接触感染等、幅広い可能性を考慮する必要があると

考えられる。なお国内において水道を介した感染はこれまで報告されていない。

今回、ヒト臨床例に関する遺伝子診断ならびに野生動物のニホンカモシカにおける原虫類調査を行った結果では、国内にて感染を起こした *G.lamblia* の遺伝子型 Assembly には A1、A2 ならびに B4 があることが明らかとなった。A2 はヒト特異的であり、HIV 陽性患者から検出されたことは、ホモセクシャルグループにおける STD 感染、原虫伝播の可能性を強く示唆するものである。A1 ならびに B4 は人畜共通感染型であるが、これまでの我々の調査で A1 は一般健康診断で検出されたもので、B4 は今回の調査で感染が確認された。国外感染例の多くが A1 であること（遠藤ら 2005）、様々な品種の飼育イヌにおいて A(1) が検出されること、あるいは野生ニホンザルより B4が検出されること（Itagaki ら 2005）、さらに今回の調査で野生ニホンカモシカからは A1 が検出されることなど、ヒト集団あるいは環境の *G.lamblia* による汚染を考える上で重要なデータが蓄積されつつある。分子疫学情報は集積によるデータベース化とその共有化が重要である。野生および飼育動物に関しては、感染源としての調査の重要性が指摘される。河川等の水系汚染における汚染実態とともに、今後も調査継続していくことが必要である。

ジアルジアに限らず、第 5 類の原虫性疾患の報告数は国内の実態を表しているとは言い難く、その部分的な状況が把握されているのが現状と思われる。その主たる要因は、これら原虫類の検査法にあることが指摘される（渡邊ら 2005）。より簡便かつ迅速な検査法は、報告数を増加させ、統計を実態に近づけさせることにつながる。今回検討した免疫クロマト法は、確定診断に有用な方法として用いられている蛍光抗体染色法あるいは ELISA 法と比較して、顕微鏡や分析装置等の設備を必要とせず、また個人（患者）単位で短時間に検査可能という利点があることは確かで、経済性、コストの問題を解決すれば普及が望まれる方法である。今回の感度試験では、ジアルジアに関しては高感度な蛍光抗体染色法とほぼ同等の性能があることが示された。クリプトスポリジウムに関しては、臨床材料を用いた試験で検出能力は確認されたが、ヌードマウス産生のオーシストに対する反応性が著しく低かったことを考えると、*C.parvum* の株による反応差を再検討すべきであると考えられる。病院等医療現場でのスクリーニング的試用、特に原因不明の下痢症例に対して、免疫クロマト法の有効性のデータを取ることを次年度の課題とする。

E．参考文献

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F．健康危機管理情報

なし

G．研究発表

なし

H．学会発表

なし

I．知的財産権の出願・登録状況

なし