ELSEVIER

Domino Liver Transplantation in Living Donors

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ABSTRACT

Domino liver transplantation (DLT) has been developed as a method to expand the donor pool. In living donors DLT, the prime concern is to avoid any disadvantage to the donor and the first recipient. Seven DLTs were performed among 211 patients who underwent living donor liver transplantation. The domino recipients included six with hepatocellular carcinoma and one with citrullinemia. The domino grafts were obtained from patients with familial amyloid polyneuropathy (FAP) including the left liver in three cases and the right liver in four. Among the seven domino recipients, a 64-year-old woman with advanced hepatocellular carcinoma died of lung metastasis. The other six domino recipients are alive without FAP symptoms. In living donor liver transplantation, because the vessels of the graft from the first donor are not long enough for anastomosis, the hepatic vessels must be left as long as possible when removing the liver from the FAP patients in order to ensure sufficient safety for vascular reconstruction. With careful decision making during the procedure, such as where to divide the vessels in the FAP patients, DLT may help address the shortage of liver grafts.

DOMINO LIVER TRANSPLANTATION (DLT) has been developed as a method to expand the donor pool. In living donors DLT,[1] the prime concern is to avoid any disadvantage to the donor and the first recipient, most of whom are patients with familial amyloid polyneuropathy (FAP). From this view, we verified the feasibility of various types of DLT in living donor liver transplantation, including consideration of vascular reconstruction in the domino recipients.

PATIENTS AND METHODS

Seven DLTs were performed among the 211 patients who underwent living donor liver transplantation between June 1990 and July 2004. All the domino donors were patients with FAP. The domino (second) recipients were selected from among adult patients with advanced hepatocellular carcinoma or acute liver failure (including acute hyperammonemia due to metabolic disorders) who had no

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Table 1. Clinical Profiles of the Domino Recipients

Patient	Indication	Graft	GV/SV(%)	Outcome
1. 45(M)	HCC + LCC	Right	46	Alive (52M)
2. 64(F)	HCC + LCC	Left	50	Dead* (22M)
3. 61(M)	HCC + LCB	Right, 2nd	34	Alive (37M)
4. 52(F)	HCC + LCB	Right	50	Alive (33M)
5. 32(M)	Citrullinemia	Left (APOLT)	26	Alive (19M)
6. 56(M)	HCC + LCC	Left	30	Alive (14M)
7. 57(M)	HCC + LCC	Right	52	Alive (13M)

HCC, hepatocellular carcinoma; LCC, hepatitis C cirrhosis; Right, right hepatic lobar graft; Left, left hepatic lobar graft; LCB, hepatitis B cirrhosis; 2nd, delayed domino transplantation; APOLT, auxiliary partial orthotopic liver transplantation.
*Died of HCC recurrence to the lung.

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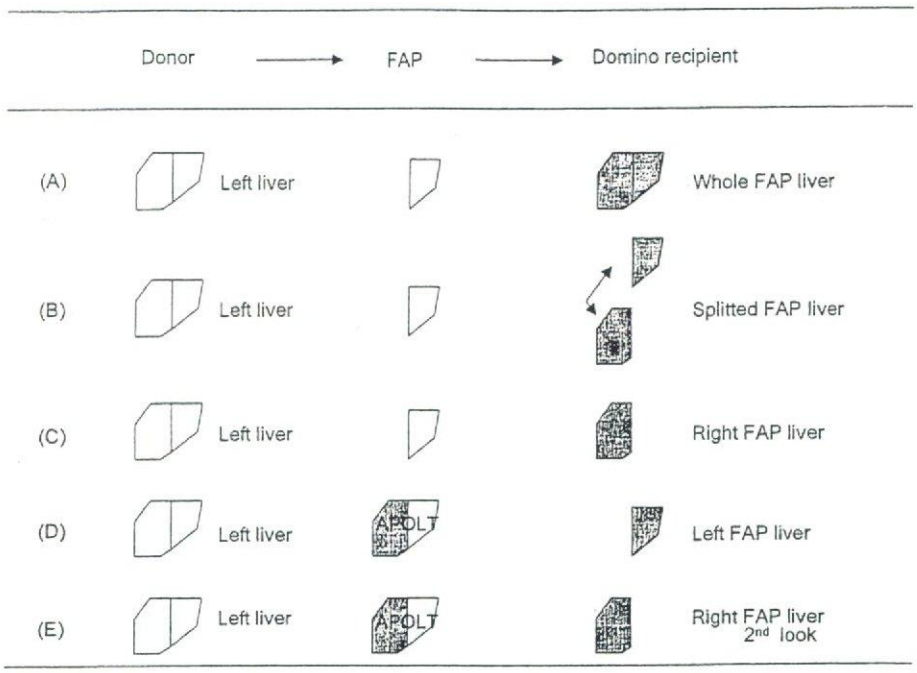

Fig 1. Possible options of DLT in living donors. Because temporary auxiliary partial orthotopic liver transplantation is feasible for patients with FAP, transplantation using the right liver for FAP is invalid from the viewpoint of donor safety.

chance of undergoing cadaveric or living related donor liver transplantation. Because there is no systematic waiting list for DLT in Japan, recipient selection is decided by each individual institution. Our procedure has been approved by the institutional ethics committee. The domino recipients included six patients with hepatocellular carcinoma and one patient with citrullinemia, aged between 32 and 64 years (Table 1). Based on the considerations for possible DLT options in living donors (Fig 1), we selected an appropriate procedure in each case. The domino grafts from the patients with FAP were the left liver in three cases, and the right liver in four. In one patient with hepatocellular carcinoma, we applied DLT using the remnant liver of a temporary auxiliary orthotopic liver transplant recipient as a liver graft for another patient at 2 months after the first liver transplantation for the FAP patient.[2] In addition, in DLT for the patient with citrullinemia, the small domino left liver was transplanted as an auxiliary orthotopic graft.[3] In the three domino recipients who received a left liver, the portal vein was reconstructed using a venous graft. In the four domino recipients with a right liver graft, a venous patch was applied for the hepatic venous reconstruction to obtain a sufficient width of the anastomosis.[4]

RESULTS

All first donors recovered well, returning to a normal life. Among the seven FAP patients, a 38-year-old woman who received a left liver graft from her husband died of hepatic artery and portal venous thrombosis. This patient showed

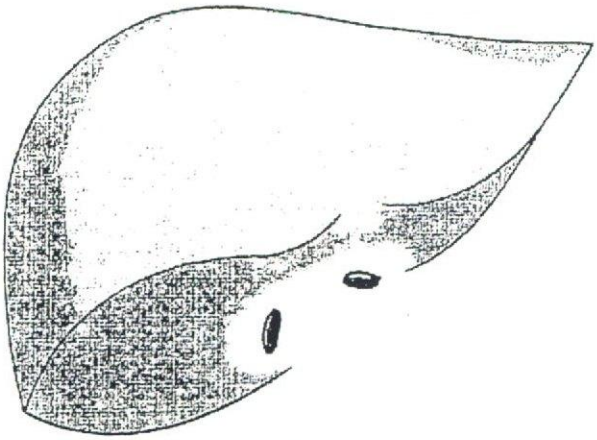

Fig 2. Whole liver graft for DLT. To ensure a sufficient level of safety for recipients with FAP, the graft has vessels of insufficient length and with multiple vascular orifices. This makes the domino transplant procedure technically complex.

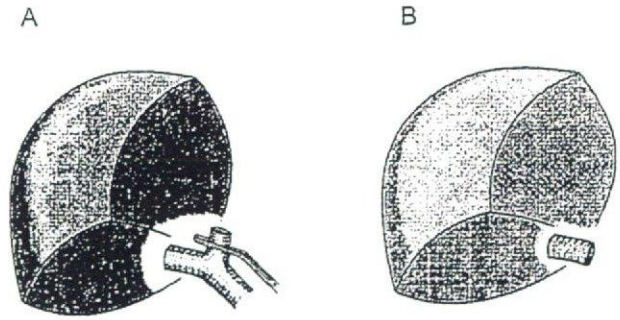

Fig 3. Left liver graft for a patient with FAP and right liver graft for a domino recipient. In the patient with FAP, the left liver is removed and the left liver graft from the living donor is transplanted orthotopically. During this phase, the right portal vein is patent and acts as a temporary portosystemic shunt to avoid portal congestion **(A)**. Thereafter, the right liver, which has sufficient length **(B)**, is removed for the domino transplant.

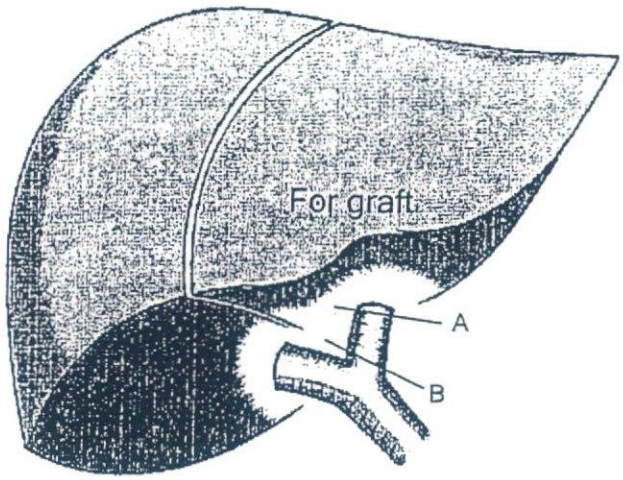

Fig 4. Left liver graft for a patient with FAP and left liver graft for a domino recipient. When performing auxiliary partial orthotopic liver transplantation for a patient with FAP, the left liver can be used for a domino graft. In this setting, division of the left portal vein at line B is not safe for vascular reconstruction in the patient with FAP. Line A is appropriate, and reconstruction with a vascular graft is necessary for the domino transplant.

extreme hypercoagulability during transplantation and did not recover even after heparinization and reanastomosis. Whether any relationship existed between the hypercoagulability and the domino transplant is unlikely. Among the seven domino recipients, a 64-year-old woman with advanced hepatocellular carcinoma died of lung metastasis at 22 months after transplantation. The other six domino recipients are alive without FAP symptoms after 13 to 52 months (median: 29 months).

DISCUSSION

The main concern in DLT is to ensure the safety of both the FAP patient and the living donor. Because the vessels of the living donor liver transplant from the first donor are not long enough for anastomosis, it is necessary to leave the hepatic vessels as long as possible when removing the liver from the FAP patient to ensure safety for vascular reconstruction. This makes the vessels attached to the domino graft very short with multiple orifices (Fig 2), thus increasing the technical complexity of vascular reconstruction in the domino recipient. From this viewpoint, a whole liver is not an ideal domino graft. Instead, when the FAP patient receives a left liver graft from the first donor and the patient's right liver is harvested as a domino graft, the domino graft vessels can be obtained to a certain extent (Fig 3). On the other hand, when the FAP patient receives a left liver graft from the first donor as auxiliary partial orthotopic liver transplantation and the patient's left liver is harvested as a domino graft, the domino graft vessels should be obtained short to maintain the FAP patient's safety (Fig 4). In this setting, a vascular graft has to be obtained for reconstruction. With careful decision making during the procedure, such as where to divide the vessels in the FAP patients, DLT may help address the shortage of liver grafts.

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HELICOBACTER

Roles of *virD4* and *cagG* genes in the *cag* pathogenicity island of *Helicobacter pylori* using a Mongolian gerbil model

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Background and Aims: The roles of the *virD4* and the *cagG* genes in the *cag* pathogenicity island of *Helicobacter pylori* for gastroduodenal pathogenesis are unclear and their roles in vivo have not been examined.

Methods: Seven week old male Mongolian gerbils were inoculated with the wild type *H pylori* TN2GF4, its isogenic *virD4*, or *cagG* mutants. Animals were sacrificed at 4, 12, and 24 weeks after inoculation. Gastric inflammation and *H pylori* density were evaluated by histology, inflammatory response (as measured by interleukin (IL)-1β mRNA levels), proliferative activity (as assessed by 5'-bromo-2'deoxyuridine labelling indices), and host systemic reaction (as measured by anti-*H pylori* IgG antibody).

Results: Degree of gastric inflammation, proliferative activity, and mucosal IL-1β mRNA levels remained low throughout the first 12 weeks in gerbils infected with the *virD4* mutants. Degree of gastric inflammation and proliferative activity increased at 24 weeks with the *virD4* mutants reaching levels comparative with those seen at four weeks with the wild-type strains. Mucosal IL-1β mRNA levels were also increased at 24 weeks with the *virD4* mutants and levels at 24 weeks were similar between the wild-type and *virD4* mutants. In contrast, gerbils infected with the *cagG* mutants had reduced ability to colonise gerbils, and no or little gastric inflammation or proliferative activity was observed.

Conclusions: Loss of the *virD4* gene temporally retarded but did not abrogate gastric inflammation. Loss of the *cagG* gene abolished gastric inflammation partially via reduced ability to colonise gerbils. Unknown factors related to the type IV secretion system other than CagA may influence gastric inflammation.

The presence of the *cag* pathogenicity island (PAI) in *Helicobacter pylori* is associated with increased mucosal inflammation and an increased risk of the development of gastric cancer or peptic ulcer disease.[1–4] The *cag* PAI is a 40 kbp cluster of approximately 27 genes that encodes a type IV secretary apparatus (a molecular syringe) which injects the CagA protein and possibly other unknown proteins into eukaryotic cells.[5–11] Defining the roles of the various genes in the *cag* PAI in the pathogenesis of *H pylori* related diseases is an area of active research interest. In vitro experiments using gastric cancer cells cocultured with *H pylori* indicate that several genes in the island are involved in induction of a proinflammatory cytokine; interleukin (IL)-8 (for example, *cagE* but not *cagA*).[3 12] IL-8 is a potent neutrophil chemotactic and activating peptide produced by gastric epithelial cells and is thought to play a major role in the pathogenesis of *H pylori* associated diseases. Recent in vivo studies using Mongolian gerbils (*Meriones unguiculatus*) showed that *cagE* knockout mutants were associated with reduced gastric inflammation[13–15] and did not induce gastric ulcers or gastric cancer.[14] In contrast, *cagA* knockout mutants caused gastric inflammation similar to the parental strain.[16] The in vivo function of other genes in the *cag* PAI has not been examined. This study therefore involves two genes in the *cag* PAI (*virD4* and *cagG*), both of which have been suggested to play unique roles based on in vitro studies,[12 17 18 19] but their roles in vivo have not been examined.

virD4 (*hp0524*; *hp* number from GenBank: AE000511) is one of seven genes in the *cag* PAI that are virulent (*vir*) gene homologues.[20] *virD4* is a key component of the type IV secretion system. In the plant pathogen *Agrobacterium tumefaciens*, VirD4 is thought to mediate introduction of the nucleoprotein complex into the transporter by an energy dependent mechanism.[21 23] In *H pylori*, VirD4 is thought to act as an adapter protein for the transfer of CagA protein and possibly other unknown proteins into the transfer channel formed by other Vir proteins in the *cag* PAI.[17] This is based on previous reports showing that knockout of the *virD4* gene resulted in loss of CagA translocation/phosphorylation as well as loss of *H pylori* induced host cytoskeletal rearrangement.[17] Although the role of VirD4 in relation to IL-8 secretion from host cells remains unclear,[12 17 18] the consensus is that loss of VirD4 does not parallel the reduction in IL-8 in contrast with other Vir factors in the *cag* PAI.

The second gene we examined was the *cagG* gene which is not a *vir* homologue gene but has weak homology to the flagellar motor switch protein gene or toxin coregulated pilus biosynthesis protein gene.[3 20] The *cagG* gene has recently been reported to be involved in adherence to gastric epithelial cells.[19] As the roles of these two gene have not been investigated in vivo, we used the Mongolian gerbil model to examine their functions in vivo in relation to gastric mucosal inflammation.

MATERIALS AND METHODS

Bacterial strains

We used a clinical isolate of *H pylori* strain TN2GF4 (kind gift from Masafumi Nakao, Takeda Chemical Industries Ltd,

Abbreviations: AI, arbitrary index; BrdU, 5'-bromo-2'deoxyuridine; CFU, colony forming units; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; MNC, mononuclear cells; PAI, pathogenicity island; PCR, polymerase chain reaction; PMN, polymorphonuclear cells, RT, reverse transcription; vir, virulent

Osaka, Japan) and its isogenic knockout mutants for *cagG* and *virD4*. Strain TN2GF4 was isolated from Japanese gastric ulcer patients and is reported to induce gastric ulcer and gastric cancer in gerbils over 62 weeks.[13]

Isogenic mutant strains were constructed from a single colony from stock frozen *H pylori*. A portion of the genes encoding the *cagG* and *virD4* genes was amplified by polymerase chain reaction (PCR) and the amplified fragment was inserted into the *Eco*RV restriction enzyme site of pBluescriptSK+ (Stratagene, La Jolla, California, USA). A chloramphenicol resistance gene cassette (a gift from DE Taylor, University of Alberta, Edmonton, Canada) was inserted into *Bsm*I and *Hind*III sites of the insert DNA for the *cagG* and *virD4* genes, respectively. All plasmids (1–2 μg) were used for inactivation of chromosomal genes by natural transformation, as previously described.[22] Inactivation of the genes was confirmed by PCR amplification followed by Southern blot hybridisation.

IL-8 levels from gastric cancer cells cocultured with *H pylori*

In vitro IL-8 measurement was performed as previously described.[23] Briefly, the human gastric cell line MKN 45 (Japanese Cancer Research Bank, Tsukuba, Japan) (1×10^5/ml) was plated onto 24 well plates and cultured for two days. *H pylori* was added to the cultured cells (bacterium to cell ratio of 100:1) and incubated for 24 hours. IL-8 in the supernatant was measured by an enzyme linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota, USA) in triplicate.

Animal, housing, and H pylori challenge

Specific pathogen free seven week old male Mongolian gerbils (MGS/Sea; Seac Yoshitomi, Fukuoka, Japan) were used in this study. They were housed in an air conditioned biohazard room designed for infectious animals, with a 12 hour light/12 hour dark cycle. They were provided with a rodent diet and water ad libitum. All experimental protocols were approved by the Animal Experiment Committee of Shinshu University School of Medicine, Matsumoto, Japan.

H pylori were grown in Brucella broth supplemented with 10% (vol/vol) horse serum for 40 hours at 37°C under microaerobic conditions and saturated humidity, with shaking at 150 rpm. After fasting for 24 hours, each animal was orogastrically inoculated with 1.0 ml of an inoculum preparation of *H pylori* (10^8 colony forming units (CFU)/ml) or sterile Brucella broth (as an uninfected control) using gastric intubation needles. No specific pretreatments (for example, acid inhibition or antibiotics) were used before orogastric *H pylori* inoculation. Four hours after administration, animals were again allowed free access to water and food.

Time course and euthanasia

Mongolian gerbils were assigned to one of three groups: inoculated with the wild-type *H pylori* strains, with its *cagG* mutants, or with its *virD4* mutants. Infected gerbils were killed and underwent necropsy at 4, 12, and 24 weeks after *H pylori* inoculation. Eight to nine gerbils were used for each time point. Uninfected control gerbils were killed at 11, 19, and 31 weeks of age (to serve as controls for the infected animals 4, 12, and 24 weeks after *H pylori* inoculation) (n = 6 each). Thirty minutes before being killed, gerbils were given 200 mg/kg of 5'-bromo-2'deoxyuridine (BrdU) intraperitoneally.

At necropsy, stomachs were opened along the greater curvature, beginning at the gastro-oesophageal junction and ending at the proximal portion of the duodenum, and observed macroscopically. Stomachs were then divided longitudinally into two parts and one half was fixed in 20% phosphate buffered formalin fixative for histological examination. The other part was further divided into the pyloric gland mucosa (antrum) and the fundic gland mucosa (corpus). The gastric mucosa was separated as much as possible from the underlying muscle using sharp dissection. Each specimen was placed on dry ice and stored at −80°C for cytokines mRNA analysis.

Table 1 Primers and probes used in this study

GAPDH	
Forward: 5'- CATGGCCTTCCGAGTTCCT-3'	
Reverse: 5'-TTCTGCAGTCGGCATGTCA-3'	
Probe: 5'-VIC-CCCCCAACGTGTCTGTCGTGGA-TAMURA-3'	
IL-1β	
Forward: 5'-GGTGACACAAGCAGCAACAAA-3'	
Reverse: 5'-CATCACACAGGACAGGTACAGATTCT -3'	
Probe: 5'-FAM-TACCGGTGGCCTTGGGCCTCA-TAMURA-3'	

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; FAM, 6-carboxyfluorescein; TAMURA, 6-carboxy-N, N, N', N'-tetramethylrhodamine.

H pylori cultures

A 1 mm^2 piece of gastric mucosa from the pyloric part of the stomach was used for culture of *H pylori*. These fragments were minced with Brucella broth and several diluted aliquots were spread on commercially available *H pylori* selective agar plates (Eiken Chemical Co., Tokyo, Japan). Cultures were incubated for seven days and the number of *H pylori* colonies per plate was counted.

Histological examination

Half of the stomach was stapled onto paper and fixed in 20% phosphate buffered formalin for 24 hours at 4°C. The fixed gastric tissue was processed for histopathological examination, and paraffin embedded sections were sliced and stained with haematoxylin-eosin or May-Grunwald-Giemsa. The degree of inflammation was graded according to the updated Sydney system.[24]

Analysis of IL-1β mRNA expression by real time quantitative PCR

Total RNA was extracted from the gastric mucosa using an RNA extraction kit (Isogen; Nippon Gene, Tokyo, Japan). After DNase treatment, 5 μg of total RNA were subjected to reverse transcription (RT) using 200 U of Moloney murine leukaemia virus reverse transcriptase (Life Technologies, Inc., Gaithersburg, Maryland, USA). Partial gerbil specific IL-1β cDNA sequences were recently cloned in our group (GenBank accession number AB164705) and we normalised IL-1β mRNA levels to the gerbil specific glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA identified previously.[13] Specific primers and TaqMan probes are listed in table 1. Real time PCR was performed using an ABI Prism 7700 Sequence-Detection System (Perkin-Elmer Applied Biosystems) at 50°C for two minutes, 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds and 60°C for 60 seconds. IL-1β mRNA levels were expressed as the ratio of IL-1β mRNA to GAPDH mRNA (100 000 × IL-1β mRNA (unit/μl)/GAPDH mRNA (unit/μl)). Each assay was performed in triplicate.

Serology

Before the animals were killed, blood samples were obtained from the orbital plexus using haematocrit tubes. Sera were used to measure the titre of anti-*H pylori* IgG antibody, as previously described.[25 26] Antibody titre was expressed as an arbitrary index (AI) with values greater than 1.37

Table 2 Prevalence of *H pylori* colonisation in gerbils evaluated with different methods

	Wild-type			*cagG* mutants			*virD4* mutants		
	4W (n=8)	12W (n=8)	24W (n=8)	4W (n=8)	12W (n=8)	24W (n=9)	4W (n=8)	12W (n=8)	24W (n=9)
Culture (pyloric)	88%	100%	88%	0%	75%	11%	25%	88%	100%
Serology	75%	100%	100%	13%	0%	22%	100%	100%	100%
Histology (pyloric)	100%	100%	100%	38%	88%	100%	100%	75%	100%
Histology (fundic)	100%	100%	100%	38%	88%	89%	88%	88%	100%
Colonisation (total)	100%	100%	100%	38%	100%	100%	100%	100%	100%

W, weeks post infection.
Gerbils were classified as *H pylori* positive if culture and/or histology yielded positive results.

(≤15 weeks of age) or 1.90 (>15 weeks) being scored as positive for *H pylori* based on our system.[26]

Statistical analyses

Results are presented as medians when the data were not distributed normally, and mean (SEM) when they were. Statistical analyses included the Student's *t* test or the Mann-Whitney rank sum test, depending on whether the data were normally distributed. Prevalence of infection was analysed using Fisher's extract test. A p value of <0.05 was considered significant.

RESULTS

In vitro IL-8 production from MKN45 cells cocultured with *H pylori*

The wild-type strain (TN2GF4) containing a complete set of the *cag* PAI genes induced greater secretion of IL-8 from MKN45 cells (mean (SEM) 3162 (147) pg/ml) than the *virD4* mutants (2318 (43) pg/ml) ($p<0.01$) or the *cagG* mutants (325 (9) pg/ml) ($p<0.001$). In agreement with reports by Selbach and colleagues,[17] the *virD4* knockout mutants induced intermediate levels of IL-8 whereas the *cagG* mutants induced less than one tenth of IL-8 produced by the wild-type strain (control IL-8 levels without *H pylori* infection were 113 (5) pg/ml).

Establishment of *H pylori* infection in Mongolian gerbils

Ninety two gerbils were used. Bacteriological, histological, and serological examination showed no detectable *H pylori* in control gerbils. Infection status in inoculated gerbils was assessed using bacteriological and histological examination (table 2). Gerbils were classified as *H pylori* positive if culture and/or histology yielded positive results. With the exception of five gerbils infected with the *cagG* mutants for four weeks, all gerbils were successfully infected (table 2). The five gerbils with failed infection were excluded from further analyses.

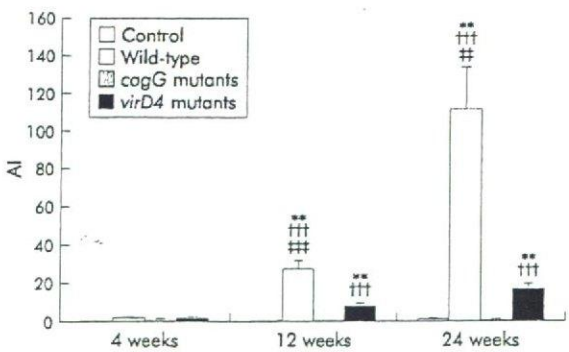

Figure 1 Titre of serum anti-*Helicobacter pylori* IgG antibodies of Mongolian gerbils inoculated orally with *H pylori* or without *H pylori* infection (control). Mean (SEM) values are presented. **p<0.01 compared with control; †††p<0.001 compared with the *cagG* knockout mutants; ‡‡p<0.01, ‡‡‡p<0.001 compared with the *virD4* knockout mutants. AI, arbitrary index.

H pylori IgG antibody titres were significantly increased in gerbils inoculated with the wild-type strains at 12 and 24 weeks compared with those at four weeks (27.9 (4.3) at 12 weeks and 111.9 (21.7) at 24 weeks compared with 2.2 (0.3) at four weeks) ($p<0.001$ for each) (fig 1). Although seroconversion occurred in all gerbils inoculated with the *virD4* mutants, antibody titres were significantly lower than those of gerbils infected with the wild-type strains (8.6 (1.2) at 12 weeks and 17.0 (3.1) at 24 weeks for the *virD4* mutants; $p<0.001$ for each). Antibody titres of gerbils infected with the *cagG* mutants were very low (maximum 3.5), and even seroconversion occurred.

Histopathological findings

Histopathological changes at 4, 12, and 24 weeks after inoculation of Mongolian gerbils with *H pylori* and in controls are shown in fig 2. Inflammatory cell infiltration in the lamina propria was negligible in controls. At four weeks after inoculation, gerbils infected with the wild-type strains showed chronic active gastritis in the antrum, with marked mucosal infiltration by neutrophilic polymorphonuclear cells (PMN) (infiltration score 1.0 (0.3)) and by mononuclear cells (MNC) (1.9 (0.3)) (figs 3, 4). At 12 weeks, with the wild-type strains, dense PMN infiltration was seen throughout the mucosa with a dense MNC infiltration in the lamina propria and submucosa in the antrum, with the normal mucosal architecture being almost completely replaced with hyperplastic epithelium (PMN 2.5 (0.2) and 0.7 (0.2); MNC 2.8 (0.1) and 1.0 (0.2) for the antrum and corpus, respectively). At 24 weeks with the wild-type strains, numerous irregularly branched dilated mucous glands were seen in the lower portion of the proper muscle layer and the PMN and MNC infiltration scores reached their maximal levels (PMN 3.0 and 1.0 (0.2); MNC 3.0 and 1.1 (0.1) for the antrum and corpus, respectively).

In contrast, gerbils infected with the *cagG* mutants showed almost no inflammation at any time after inoculation (figs 3, 4). MNC and PMC infiltration scores in gerbils infected with the *cagG* mutants were significantly lower than those with the wild-type strain throughout the observation periods.

Gerbils infected with the *virD4* mutants showed mild cellular inflammation four and 12 weeks after inoculation (MNC and PMC infiltration scores less than 0.5). MNC and PMC infiltration scores in gerbils infected with the *virD4* mutants were significantly lower than those with the wild-type strains throughout the observation periods. Interestingly, however, at 24 weeks after inoculation, gerbils infected with the *virD4* mutants showed chronic active gastritis with marked mucosal infiltration in the antrum (MNC 1.8 (0.3) and PMN 1.3 (0.3)) whereas mucosal infiltration in the corpus remained very mild (figs 3, 4). The amount of cellular infiltration in the antrum increased in gerbils infected with the *virD4* mutants at 24 weeks

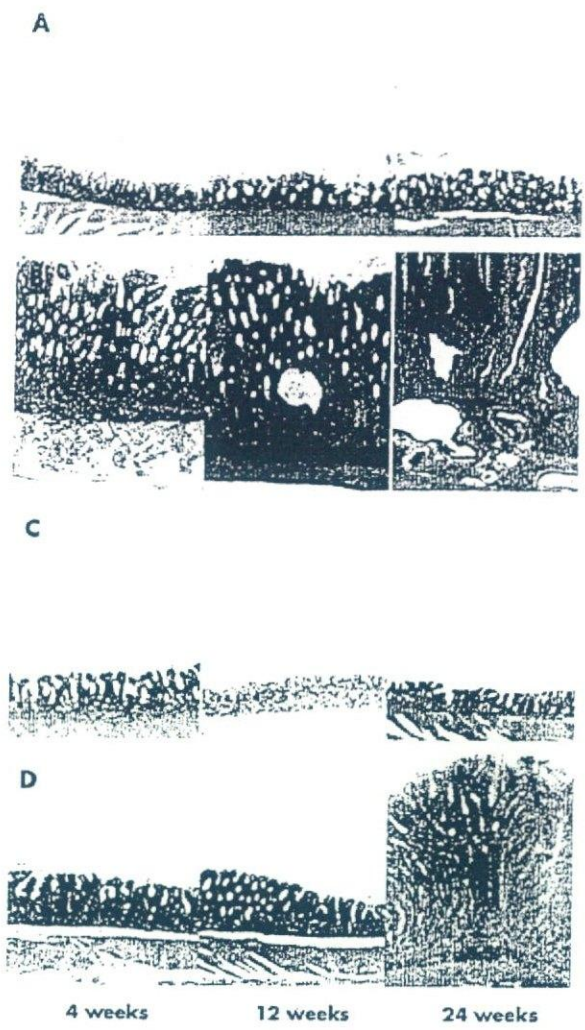

Figure 2 Histology of the gastric pyloric mucosa of (A) control, (B) wild-type *Helicobacter pylori* strain TN2GF4, (C) its isogenic *cagG* knockout mutant, or (D) *virD4* knockout mutant. Haematoxylin and eosin stain, original magnification ×200. (A) In controls, inflammatory cell infiltration in the lamina propria was negligible throughout the experimental periods. (B) In gerbils infected with the wild-type strain, pyloric mucosa showed marked infiltration by neutrophilic polymorphonuclear cells and mononuclear cells at four weeks after inoculation and the inflammatory response increased with the duration of infection. The pyloric mucosa became thickened from four weeks after inoculation, and irregularly branched and dilated mucous glands appeared at 24 weeks after inoculation. (C) In gastric mucosa infected with the *cagG* knockout mutants, inflammatory cell infiltration in the lamina propria was negligible throughout the experimental periods. (D) Pyloric mucosa of gerbils infected with the *virD4* knockout mutants showed mild inflammatory inflammation at four and 12 weeks after inoculation. At 24 weeks after inoculation, pyloric mucosa showed increased degrees of inflammatory cell infiltration and became thickened.

compared with 12 weeks, and the pyloric mucosa appeared expanded similar to that observed with the wild-type strains. The grade of mucosal inflammation observed in gerbils infected with the *virD4* mutants at 24 weeks was similar to those with the wild-type strains at four weeks.

H pylori density score, as evaluated by histology in the antrum of gerbils infected with the *virD4* mutants, was significantly greater than that in animals infected with the wild-type strains at 24 weeks (fig 5) ($p<0.01$). Importantly,

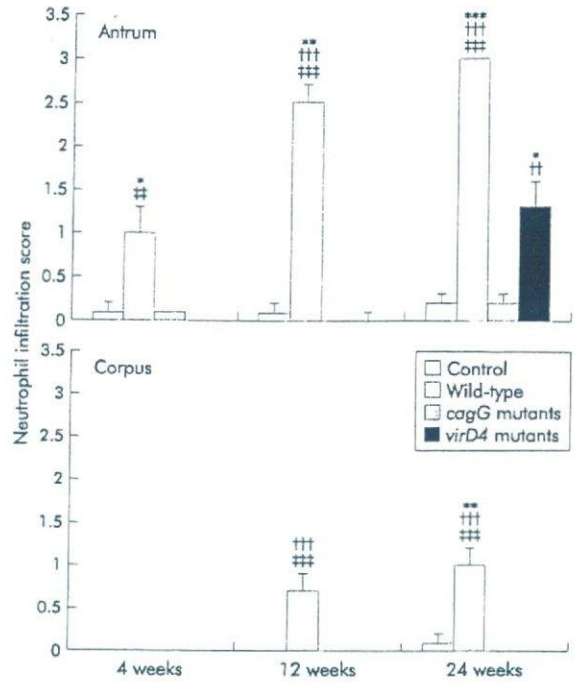

Figure 3 Neutrophil infiltration scores at 4, 12, and 24 weeks after inoculating with *Helicobacter pylori* or without *H pylori* (control). Mean (SEM) values are presented. *p<0.05, **p<0.01, ***p<0.001 compared with control; ††p<0.01, †††p<0.001 compared with the *cagG* knockout mutants; ‡‡p<0.01, ‡‡‡p<0.001 compared with the *virD4* knockout mutants.

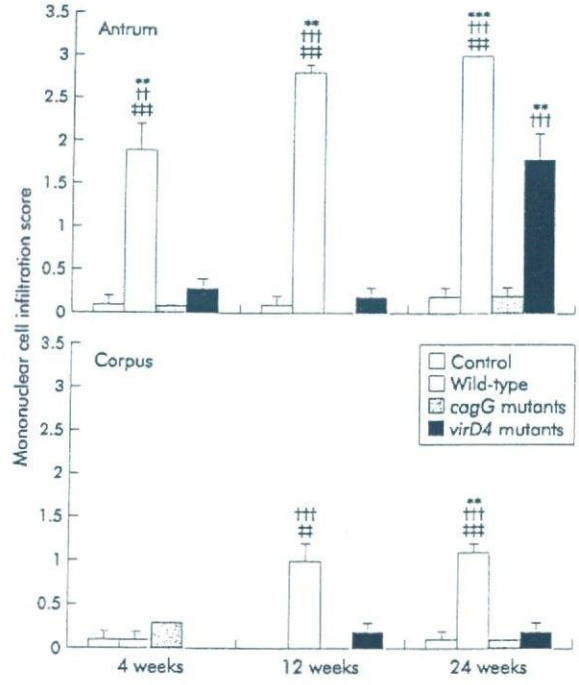

Figure 4 Mononuclear cell infiltration scores at 4, 12, and 24 weeks after inoculating with *Helicobacter pylori* or without *H pylori* (control). Mean (SEM) values are presented. **p<0.01, ***p<0.001 compared with control; ††p<0.01, †††p<0.001 compared with the *cagG* knockout mutants; ‡‡p<0.01, ‡‡‡p<0.001 compared with the *virD4* knockout mutants.

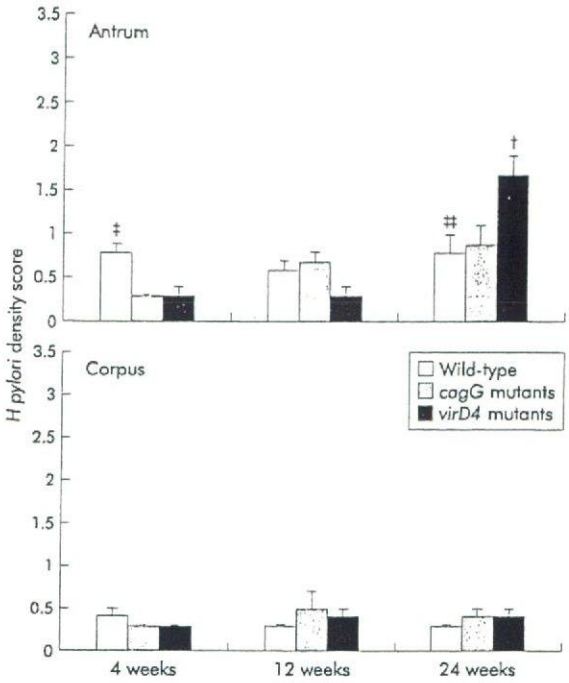

Figure 5 Scores for *Helicobacter pylori* density evaluated by histology at 4, 12, and 24 weeks after inoculation with *H pylori*. Mean (SEM) values are presented. †p<0.05 compared with the *cagG* knockout mutants, ‡p<0.05, ‡‡p<0.01 compared with the *virD4* knockout mutants.

gerbils infected with the *cagG* mutants showed no inflammation at any time after inoculation. *H pylori* density score was mostly equivalent to the wild-type strains or *virD4* mutants (fig 5).

BrdU labelling indices

Detectable BrdU labelling indices were not observed in control gerbils without *H pylori* infection. BrdU labelling indices in the antrum were independent of the duration of *H pylori* infection both in gerbils infected with the wild-type strains (mean 26.3 (3.1) to 34.6 (3.3)) and the *cagG* mutants (mean 3.2 (0.5) to 11.3 (7.3)) (fig 6). In contrast, BrdU labelling indices were significantly increased in the *virD4* mutants at 24 weeks after inoculation (29.3 (3.9) at 24 weeks compared with 6.1 (0.8) at four weeks and 3.8 (0.5) at 12 weeks) (p<0.001 for each). Overall, BrdU labelling indices were higher in gerbils infected with the wild-type strains compared with those with the *cagG* mutants or the *virD4* mutants at four or 12 weeks. Indices were also significantly higher in gerbils infected with the wild-type strains compared with those with the *cagG* mutants at 24 weeks (p<0.001) whereas the indices were similar among gerbils infected with the wild-type strains and the *virD4* mutants.

Mucosal IL-1β mRNA levels

In the control group, mucosal IL-1β mRNA levels were very low throughout the observation periods (10 000× mean (SEM); IL-1β/GAPDH 1.8 (0.4) to 6.6 (0.8)) (fig 7). At four weeks after inoculation, mucosal IL-1β mRNA levels were significantly greater in gerbils infected with the wild-type strains compared with the *virD4* or *cagG* mutants. Mucosal IL-1β mRNA levels at 12 weeks with the wild-type strains were also significantly higher than those with the *virD4* or

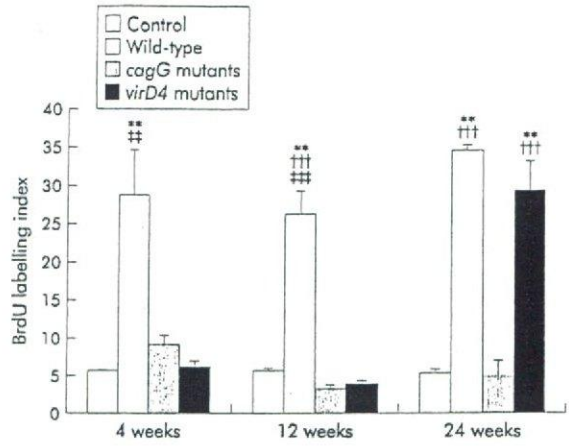

Figure 6 5′-Bromo-2′deoxyuridine (BrdU) labelling indices in the pyloric mucosa. Mean (SEM) values are presented. **p<0.01 compared with control; †††p<0.001 compared with the *cagG* knockout mutants; ‡‡p<0.01, ‡‡‡p<0.001 compared with the *virD4* knockout mutants.

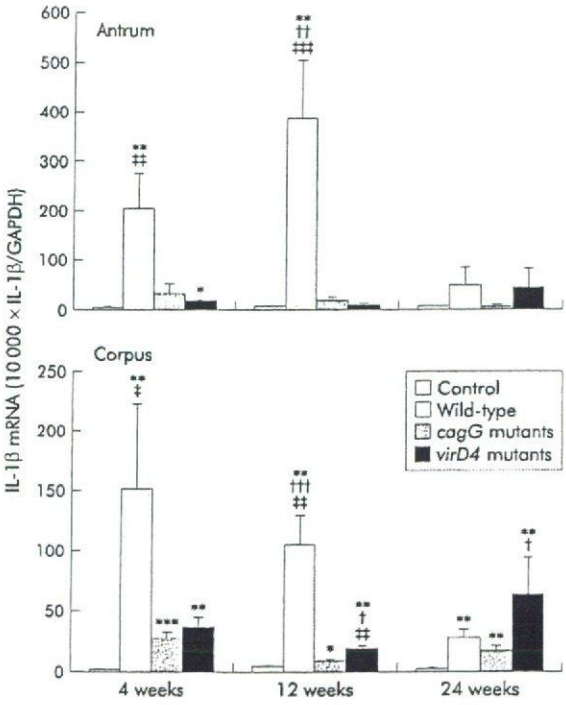

Figure 7 Mucosal interleukin (IL)-1β mRNA levels in gerbils at 4, 12, and 24 weeks after inoculating with *Helicobacter pylori* or without *H pylori* (control) in the pyloric mucosa (antrum) and fundic mucosa (corpus). Mean (SEM) values are presented. *p<0.05, **p<0.01, ***p<0.001 compared with control; †p<0.05, ††p<0.01, †††p<0.001 compared with the *cagG* knockout mutants; ‡‡p<0.01, ‡‡‡p<0.001 compared with the *virD4* knockout mutants.

cagG mutants. At 24 weeks after inoculation, IL-1β levels decreased in gerbils infected with the wild-type strains.

Mucosal IL-1β levels were very low in gerbils infected with the *virD4* mutants throughout the first 12 weeks; however, these levels tended to increase at 24 weeks (45.6 (36.0) for the antrum and 63.0 (30.6) for the corpus). In contrast, IL-1β

levels were very low in gerbils infected with the *cagG* mutants throughout the observation periods (fig 7).

DISCUSSION

We used the Mongolian gerbil model to examine the effect of two previously unstudied genes in the *cag* PAI (*virD4* and *cagG*) on gastric inflammation in vivo. Wild-type *H pylori* caused typical severe gastritis in gerbils whereas the *virD4* mutants caused very low levels of gastric inflammation, mucosal proliferative activity, and mucosal IL-1β levels throughout the first 12 weeks. *H pylori* density was similar with the different inocula, confirming that the differences were not due to bacterial load. At 24 weeks, the degree of gastric inflammation and proliferative activity in gerbils infected with the *virD4* mutants increased, reaching levels comparative with those seen at four weeks with the wild-type strains. *H pylori* density in the antrum at 24 weeks in gerbils infected with the *virD4* mutants was significantly higher than that with wild-type *H pylori* ($p = 0.03$). Lack of acute inflammation might help growth of the *virD4* mutants; however, it remains unclear whether it is sufficient to explain the results.

Mucosal IL-1β levels at 24 weeks were similar for *virD4* mutants and wild-type infections. IL-1β levels with the wild-type strains were maximal at four weeks in the corpus and at 12 weeks in the antrum. In gerbils and in mice, IL-1β mRNA levels do not mirror chronic mucosal inflammation.[27 28] In contrast, in humans, IL-1β levels are consistently elevated in *H pylori* infected gastric mucosa.[29] IL-1β mRNA levels in the corpus were very low in the chronic phase of the infection, suggesting that induction of acute inflammation rather than inhibition of gastric acid secretion[29 30] is the main role of IL-1β in gerbils. Probably the most important proinflammatory cytokine in the gastric mucosa is IL-8. Gerbils do not encode an IL-8 gene, as cross species RT-PCR techniques failed to identify an IL-8 gene (unpublished observation). We selected IL-1β based on the fact that in humans, mucosal IL-8 levels were closely correlated with mucosal IL-1β levels.[29 31] Future studies will examine cytokine expression using IL-8 families such as KC which behave like IL-8 in mice.

With the wild-type strains, the degree of gastric inflammation reached maximal levels at 12–26 weeks and proliferative activity at four weeks.[25 32 33] Loss of the *virD4* gene temporally retarded but did not abrogate *H pylori* induced gastric inflammation, and proliferative activity with the *virD4* mutants was similar to that with the wild-type strains at 24 weeks. We did not examine animals beyond 24 weeks and can only speculate regarding later time points.

The *virB4* (*cagE*) knockout mutants produce mild gastritis and not gastric ulcers.[13–15] VirB4 is a major component of the type IV secretion system such that loss of the *cagE* gene results in loss of CagA translocation/phosphorylation as well as loss of host cytoskeletal rearrangement and IL-8 induction.[3 12 17] Although the *virD4* mutants also lose the ability to translocate CagA into host cells, *cagA* mutants can produce inflammation[16] consistent with in vitro studies showing that CagA is not responsible for IL-8 induction. Lack of inflammation with the *virD4* mutants in the first 12 weeks suggests the absence of as yet unidentified factors that translocate into epithelial cells using the type IV secretory pathway or interact with the type IV secretion system. Several factors other than the *cag* PAI, in particular OipA as one of the outer membrane proteins, are related to induction of mucosal IL-8 and gastric inflammation.[22 34 35] In addition, *cag* PAI status is closely related to OipA status (for example, if the strains possess the *cag* PAI, strains almost always possess functional OipA).[34] However, possible interactions between OipA and *cag* PAI were not examined in these experiments.

The *cagG* mutants did not produce an inflammatory response or increase proliferative activity, most likely related to their poor ability to colonise gerbil gastric mucosa. The *cagG* gene is not a *vir* homologue gene and has a weak homology to the flagellar motor switch protein gene or toxin coregulated pilus biosynthesis protein gene.[3 20] The current consensus is that loss of the *cagG* gene also results in loss of CagA translocation/phosphorylation.[3 12] Recent reports suggest that isolates lacking *cagG* genes have decreased adherence to epithelial cell lines.[19] An in vivo study has shown no relationship between *cagG* and clinical outcome[36]; the population studied (Chinese) were predominantly infected with *cag* PAI positive strains such that the effect of the *cagG* gene could not be examined. Most reports, including our present study, suggest that loss of the *cagG* gene results in almost complete elimination of *H pylori* induced IL-8

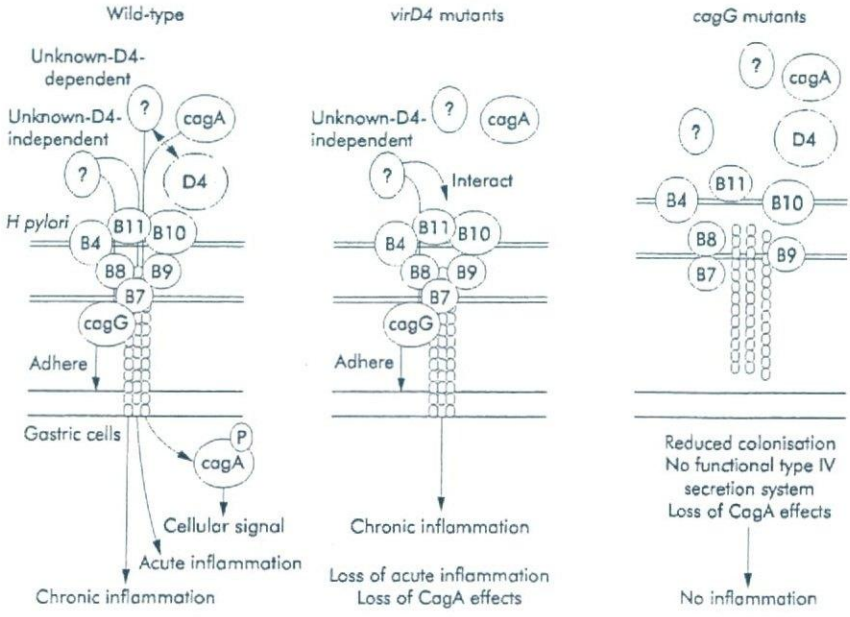

Figure 8 Hypothetical model for induction of host responses by *Helicobacter pylori*.

induction.[3 37] However, a recent report suggested that precise deletion of the *cagG* gene resulted in no reduction in IL-8 induction.[12] Gerbils infected with *cagG* mutants showed no inflammation although the *H pylori* density score was generally equivalent to wild-type strains or *virD4* mutants (fig 5). From these data it is not possible to define whether or not the lack of inflammation with *cagG* mutants is related to reduced colonisation, loss of the type IV secretion system, or both. Complementation experiments will be needed to resolve this issue.

Our current hypothetical model is presented in fig 8. VirD4 is thought to act as an adapter protein for the transfer of CagA protein and possibly other unknown proteins (D4 dependent) into the transfer channel formed by other Vir proteins in the *cag* PAI. We also hypothesise the presence of unknown proteins independent of VirD4. As *virD4* mutants are unable to translocate CagA as well as any D4 dependent factors, loss of CagA effects and loss of D4 dependent factors occurs. However, it is possible that D4 independent factors may be translocated into cells or interact with the type IV secretion system, inducing chronic inflammation. *cagG* mutants have decreased adherence to epithelial cells and reduced ability to colonise gerbils. In addition, they are unable to translocate CagA and any D4 dependent or independent factors due to loss of functional transporter system such that inflammation would not be expected.

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Conflict of interest: None declared.

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肝移植の現状と合併症*

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はじめに

肝臓はきわめて多様な機能を営む臓器であり，人の命を支えることのできる人工肝臓の完成にはなお長い年月を要する。このため末期肝不全に陥った患者を救命する唯一の方法は肝移植である。日本移植学会によるファクトブック2005[1]によると，国内で肝移植が必要とする状態にありながらも肝移植を受けることなく死亡している患者数は年間約2,200人と推計されている（表1）。

わが国における肝移植は，生体肝移植が1989年に開始され[2]，その後，諸施設で症例が重ねられつつある。しかし，これまでの15年間に計2,600例を超える症例に対して生体肝移植が施行されているものの，前述の肝移植適応患者数には遠く及んでいない現状にある（表2）。一方，脳死肝移植は，1997年に「臓器移植法」が施行され，1999年に脳死肝移植国内第1例[3]が施行されて以来，2005年6月30日の時点で28例に対して施行されてきた。

I．肝移植の適応

一般的には，進行性の肝疾患のため末期状態にあり従来の治療方法では余命1年以内と推定される患者に対して肝移植の適応を検討する。ただし，先天性肝・胆道疾患，先天性代謝異常症などについては余命がより長いと評価されても適応となりうる。具体的な適応疾患を表3に示す。適応となる年齢の上限はおおむね60歳代であり，60歳を超えて高齢になるほど合併症の発生率や死亡率が高くなる傾向にある。

II．生体肝移植の実施状況

国内では，血縁者，家族などが自分の肝臓の一部を提供する生体肝移植を中心に行われてきた。脳死肝移植が開始された後もその数が少ないため，生体肝移植の症例数は年々増加の一途をたどっている（表2）。その一方で，国内でもドナーの死亡例があり生体肝移植という医療のあり方について見直しの機運があることも事実である。

藤堂らは国内における成人生体肝移植症例についてアンケート調査を行い，集計結果を報告した[4]。それによると，1991年1月～1999年12月までの期間に，20施設で計308例の成人生体肝移植が行われた。原疾患は，胆汁うっ滞性肝疾患129例（42%），ウイルス性肝硬変をはじめとする慢性肝細胞障害62例（20%），劇症肝不全54例（17%），代謝性肝疾患43例（14%），悪性腫瘍21例（7%）であった。

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* Liver transplantation：current status and postoperative complications

key words：living donor liver transplantation, cadaveric liver transplantation

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表 1　肝移植適応患者数の概算（年間）

疾　患	発生数	適応者数
胆道閉鎖症	140	100
原発性胆汁性肝硬変	500	25
劇症肝炎	1,000	100
肝硬変	20,000	1,000
肝細胞癌	20,000	1,000
合　計		約 2,200

表 3　肝移植の適応疾患

1．劇症肝炎
2．先天性肝・胆道疾患
3．先天性代謝異常症
4．原発性胆汁性肝硬変症
5．原発性硬化性胆管炎
6．肝硬変（肝炎ウイルス性，二次性胆汁性，アルコール性，その他）
7．肝細胞癌
8．その他

表 2　日本における脳死肝移植，生体肝移植数

年	'89	'90	'91	'92	'93	'94	'95	'96	'97	'98	'99	'00	'01	'02	'03	'04	合計
生体	1	10	30	31	51	82	111	120	157	208	250	327	417	432	440	550	3,217
脳死											2	6	6	7	2	3	26

III．生体肝移植におけるドナーの術後経過

ドナーの術後経過に関する調査は一部の施設で行われ，その結果が報告されてきた[5]。生体肝移植ドナーの術後経過に関する調査結果を公表していくことは重要とされる一方，合併症として扱う基準の差などに起因する施設別調査の限界があり，系統的な全国調査の必要性が指摘されてきた。

日本肝移植研究会ドナー調査委員会（委員長：里見 進・東北大学教授）は，わが国における2003年12月末で2,667例の生体肝移植症例の全ドナーを対象として，健康状態や心理状態などを把握するための調査を行った[6]。その調査結果の要点は以下のとおりである。

1）術後経過：回答者の手術前の予想と比較すると，術後経過は「順調だった」909名（61.6%），「どちらともいえない」364名（24.7%），および「悪かった」203名（13.8%）であった。

2）術後の入院期間：「10日以内」309名（21.1%），「2週間以内」498名（33.9%），「3週間以内」307名（20.9%），「1カ月以上」118名（8.3%）であり，成人症例に対するドナーの入院期間は，小児症例に対するドナーよりも長い傾向がみられた。

3）術後に生じた症状：術後経過期間別にみた術後の症状を表3に示す。手術創に関する症状と消化器系の症状の頻度が高く，また，術後4カ月～1年までの間に外来通院および入院を要したドナーは，それぞれ237名（16.7%），30名（2.0%）であった。

IV．生体肝移植におけるレシピエントの術後経過

日本肝移植研究会の肝移植症例登録報告[7]によると，生体肝移植症例の1年，3年，5年生存率は，それぞれ81%，78%，77%であった。

前述の成人生体肝移植の集計結果[6]では，308例中223例（72.4%）が調査時点で生存しており，疾患別の1年累積生存率は，原発性胆汁性肝硬変（n＝73）71.3%，胆道閉鎖症（n＝29）65.1%，B型ウイルス性肝硬変（n＝25）73.2%，C型ウイルス性肝硬変（n＝23）73.3%，原発性硬化性胆管炎（n＝22）80.4%，劇症肝不全（n＝19）82%であった。また，血液型不適合症例は17例で，その生存率は一致および適合例に比べて有意に不良であった（図1）。この集計におけるレシピエントの術後

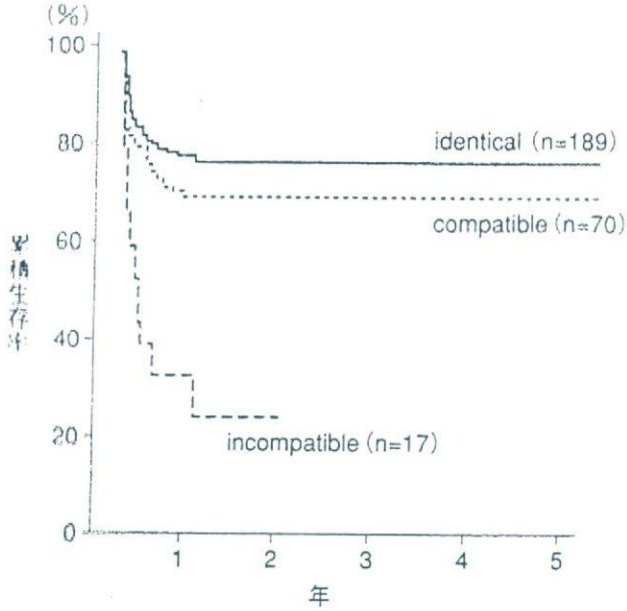

図 1　成人生体肝移植における ABO 血液型適合性と生存率

血液型不適合（incompatible）症例の生存率は，適合（compatible），一致（identical）症例に比べて有意に不良である。（文献 4）より引用）

表 4　成人生体肝移植レシピエントの術後合併症

合併症	数	%
術後出血	68	22.1
胆道合併症		
縫合不全	25	8.1
吻合部狭窄	16	5.2
その他	7	2.3
血管合併症		
血栓症	17	5.5
吻合部狭窄	15	4.9
primary nonfunction	1	0.3

（文献 4）より引用）

表 5　成人生体肝移植レシピエント死亡例（n＝85）の死亡原因

原因	数	%
敗血症	45	52.9
出血	12	14.1
血管合併症	12	14.1
原疾患再発	2	2.3
その他	20	23.6

（文献 4）より引用）

合併症および死亡原因を**表 4, 5** に示す。肝移植後には，術前から認められた腎機能障害（肝腎症候群），免疫抑制薬（タクロリムス，シクロスポリン）の副作用による腎機能障害が問題となることがあり，一時的に血液透析を必要とする場合があるが，その頻度は高くなく，そのほとんどが可逆的である。ただし，クレアチニン-クリアランスの低下を認め，腎で代謝される薬剤の投与量調節を必要とすることを 10～20%の頻度で経験する。生体肝移植の術後生存率を**図 2** に示す。

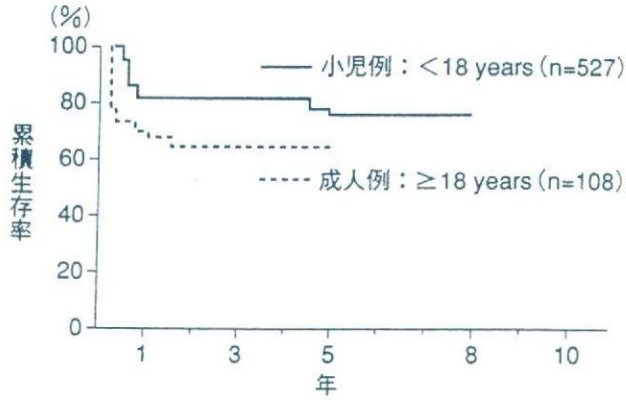

図 2　生体肝移植の術後生存率（文献 4）より引用）

V．生体肝移植の費用

生体肝移植については，2004 年 1 月 1 日より健康保険の対象となる疾患が大幅に拡大され，患者にとって福音となった。現在の適応疾患は，先天性胆道閉鎖症，進行性肝内胆汁うっ滞症（原発性胆汁性肝硬変と原発性硬化性胆管炎を含む），アラジール症候群，バッドキアリー症候群，先天性代謝性疾患（家族性アミロイドポリニューロパチーを含む），多発性嚢胞肝，カロリ病，肝硬変（非代償期）および劇症肝炎（ウイルス性，自己免疫性，薬剤性，成因不明を含む）と定められている。また，肝硬変に肝細胞癌を合併している場合には，遠隔転移と血管侵襲を認めないもので，肝内に直径 5 cm 以下 1 個，または 3 cm 以下 3 個以内が存在する場合に限られている。これら以外の場合は保険が適応されず，原則的に患者の自己負担となる。

VI. 脳死肝移植の実施状況

日本臓器移植ネットワークによると，2005年6月30日の時点で86人が脳死肝移植を希望して待機中である。先に述べたように，肝移植が必要な患者は余命が1年以内と推定されており，待機期間が長期にわたると，残念ながら死を迎える現状にある。表1，2のデータから，年間2,000人弱の患者が肝移植の適応がありながら亡くなっているものと推定される。過去に脳死肝移植を希望して日本臓器移植ネットワークに登録した人のうち，2005年3月31日の時点ですでに134人が死亡している。その他，10人が海外に渡航して肝移植を受け，90人が生体肝移植を受けた。全体でみると，脳死肝移植を希望して登録した人のうち，実際に脳死肝移植を受けることができた人は7%にすぎないのが現状である。また，国内で脳死肝移植を受けた人の移植までの待機期間は平均490日であった。

なお，脳死肝移植実施施設は，大阪大学，岡山大学，九州大学，京都大学，慶應義塾大学，信州大学，東京大学，東北大学，長崎大学，名古屋大学，新潟大学，広島大学，北海道大学の13施設である（五十音順）。

VII. 脳死肝移植の成績

国内で脳死肝移植を受けた28人のうち，現在23人が生存しており，累積生存率は1年81%，3年81%，5年81%である。ちなみに肝移植後の世界最長生存例は28年である。

VIII. 脳死肝移植の費用

脳死肝移植については，認定13施設のうち一部の施設では高度先進医療が認可されており，移植手術費（施設により異なる）と臓器搬送費（100～250万円：搬送距離により異なる）が患者負担となるが，移植術後の管理費（免疫抑制薬を含む）については保険から給付される。その他の施設は高度先進医療の申請中であり，現時点では各施設がそれぞれの方針で対応している。

おわりに

生体肝移植が肝移植の大部分を占める日本の状況は，世界的にはきわめて特異である。以前から生体肝ドナーの死亡例が国外から報告されていたが，2003年には国内でも初めての死亡があった。また，肝提供後の生体ドナーには少なからぬ合併症があることも明らかにされている。現在，法改正が国会で議論されているが，これを含めて脳死臓器提供数を増やすさまざまな努力が必要と考えられる。

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信州大学における肝移植後シクロスポリンの使用経験

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Liver transplantation under ciclosporine in Shinshu-University

key words : 肝移植，免疫抑制療法，シクロスポリン，タクロリムス

シクロスポリンは強力な免疫抑制効果を有する薬剤であり，肝移植への臨床応用開始後四半世紀をすぎた現在でも，タクロリムスとともに肝移植後の免疫抑制に欠くことのできない薬剤である．

本稿では，信州大学における肝移植後のシクロスポリンの使用経験を，特に免疫抑制剤の変更という観点から報告する．

対象と方法

1990年6月～2005年6月までに，信州大学で肝移植を行った232例を対象とした．

レシピエントは18歳未満の小児111例(47.8%)，18歳以上の成人121例(52.2%)で，女性93例(40%)，男性139例(60%)である．原疾患は，胆道閉鎖80例，劇症肝炎27例，家族性アミロイドポリニューロパチー26例，C型肝炎24例，シトルリン血症11例，原発性硬化性胆管炎9例，B型肝炎7例，その他48例であった．ドナーは，患者の親129例，子46例，兄弟23例，夫婦20例，その他の血縁者3例，ドミノ7例，脳死4例で，血液型は，identical 175例，non-identical 50例，incompatible 7例であった．

シクロスポリンは1993年9月までは導入療法に用い，それ以降はタクロリムス導入例におけるレスキュー療法に用いた．シクロスポリンは投与開始時は4 mg/kg/day経静脈的持続静注で開始し，血中濃度を1日3回程度測定して，目標濃度

表1 シクロスポリンによる導入症例

症例	年齢	原疾患	急性拒絶反応	OKT-3	タクロリムスへ変更
1.	7	胆道閉鎖	+		+
2.	6	胆道閉鎖	+	+	+
3.	1	乳児肝炎	+		
4.	0.7	胆道閉鎖			
5.	12	胆道閉鎖	+		+
6.	9	胆道閉鎖	+		
7.	7	胆道閉鎖			
8.	4	胆道閉鎖			
9.	15	劇症肝炎	+	+	+
10.	8	胆道閉鎖			
11.	4	劇症肝炎	+		+
12.	0.6	胆道閉鎖			
13.	0.9	胆道閉鎖	+		+
14.	1	胆道閉鎖			
15. *	18	非B非C肝炎			

*再移植例

になるよう適宜投与量を変更した．経口摂取は腸蠕動が回復したと判断されたあとに開始するが，経静脈的投与からの切換えは経口投与における吸収率を1/3として全投与量の1/2ないし1/3ずつ切り換えた．血中濃度は経口摂取直前のC_0でコントロールした．

シクロスポリンでの導入例でもタクロリムスでの導入例でも，これらの薬剤に伴うなんらかの有害事象が発生した場合，あるいは投与継続に不都合と考えられる事象が発生した場合には，躊躇せずにもう一方の薬剤に変更した．変更の際にはタクロリムスとシクロスポリンの両者の血中濃度を測定し，タクロリムスの濃度を14倍してシクロスポリンの濃度に換算し，目標値となるようにした．

これらについて，肝移植後に使用した免疫抑制剤について検討した．

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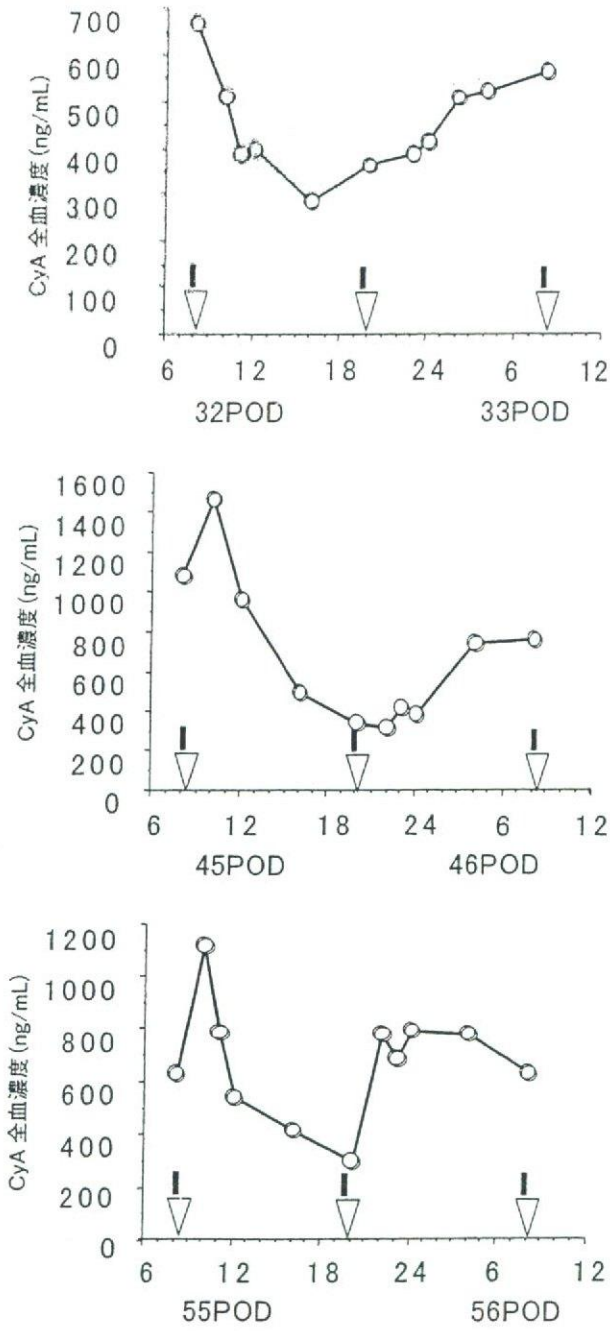

図1　劇症肝炎の15歳・男児における
シクロスポリン濃度の日内変動
↓：経口投与

結　果

1993年9月までに行われた肝移植全例14例と，シクロスポリンで免疫抑制を行っていた症例の再移植例1例の計15例でシクロスポリンが導入療法に用いられ，216例ではタクロリムスにより導入が行われ，一卵性双生児間の1例では免疫抑制療法を行わなかった．

シクロスポリンで導入した15例中8例で急性拒絶反応が認められ，うち2例ではステロイド抵抗性であったため，ムロモナブCD3(OKT-3)の投与を必要とした(表1)．シクロスポリン投与例の血中濃度は不安定であり，個体差，朝と夕方のトラフ値の差，日内変動が二峰性でないなどが認められた(図1)．

免疫抑制剤の変更は，シクロスポリンでの導入例15例のうち6例でタクロリムスへ，タクロリムスによる導入例216例のうち79例でシクロスポリンに変更された．それぞれの薬剤の変更状況を示すと，表2のように症例によっては何度も

表2　免疫抑制剤の変更状況

免疫抑制剤	例数
CyA	9
CyA → Tac	4
CyA → Tac → CyA	2
Tac	137
Tac → CyA	55
Tac → CyA → Tac	16
Tac → CyA → Tac → CyA	5
Tac → CyA → Tac → CyA → Tac	2
Tac → CyA → Tac → CyA → Tac → CyA	1

CyA：シクロスポリン，Tac：タクロリムス

表3　タクロリムスからシクロスポリンへの
変更理由

変更理由	回数	変更理由	回数
肝機能障害	20	腎機能障害	3
痙攣	13	不明	3
食欲低下	10	溶血性貧血	2
PTLD(EBV)	9	TEN	1
糖尿病	7	頭痛	1
心毒性	5	精神障害	1
下痢	5	パーキンソン	1
TTP	5	社会的	1
意識障害	5		

表4　シクロスポリンからタクロリムスへの
変更理由

変更理由	回数
拒絶反応	20
肝機能障害	12
不明	2

変更されているものもあった.

2005年6月にシクロスポリンが用いられているのは72例であった. 免疫抑制剤の変更例についてその理由を検討したところ, 表3のように, タクロリムスからシクロスポリンへ変更した理由は多岐にわたったが, シクロスポリンからタクロリムスへの変更理由は拒絶反応や肝機能障害であった(表4).

考　察

信州大学では初期にシクロスポリンによる導入療法を行っていたが, 急性拒絶反応, 特に当時の肝移植14例のうち2例でステロイド抵抗性の急性拒絶反応を経験し, また, 血中濃度が不安定であることなどを考慮して, 1993年10月以降の導入療法はタクロリムスとステロイドで行うこととした. シクロスポリンもタクロリムスも, ともに一般的に用いられている薬剤と比較して副作用の発生頻度は高く安全域が狭いため, その使用に当たっては血中濃度を測定しながら投与量を決めていく必要がある.

これら免疫抑制剤使用中に, シクロスポリンの多毛やタクロリムスの手指の振戦など以外の表3, 4に示すような症状があるときは, 副作用の可能性を考慮して, 比較的容易に免疫抑制剤を変更してきた. この結果, 使用例数自体がタクロリムス使用例ではシクロスポリン例より多いこともあるが, タクロリムスは多彩な理由でシクロスポリンに変更されていたのに対し, シクロスポリンからの変更例では, 拒絶反応と肝機能障害の二つの理由に集中していた. これはこの2項目以外の点について, シクロスポリンの安全性が高いと考えられる可能性が示される.

結　語

現在シクロスポリンは信州大学において, タクロリムス使用例に対するレスキュー療法に主に用いられているが, 拒絶反応の発生という観点を除くと安全に用いられていると考えられる. しかし, 移植片のみを免疫寛容に導くような薬剤の開発が理想である.

免疫抑制薬

中澤 勇一・宮川 眞一

免疫抑制薬血中濃度測定の臨床的意義

免疫抑制薬のうち，シクロスポリン(CyA)とタクロリムス(Tac)はT細胞でのIL-2・インターフェロンγなどのサイトカインの産生を抑制する免疫抑制剤であるが，消化管での吸収，代謝あるいは排泄が個体間・個体内で顕著に異なっているため，血中濃度をモニタリング(therapeutic drug monitoring：TDM)し，各疾患・病態ごとに設定される至適濃度を維持するように，その投与量を調節しなければならない薬剤である．

免疫抑制薬血中濃度測定の重要性

1．血中濃度測定の重要性

●血中濃度低下による臓器移植後の拒絶反応，移植片対宿主病(graft - versus - host disease：GVHD)の発症を予防する．

●過剰な血中濃度上昇による腎機能障害・中枢神経症状・高K血症・高血圧など濃度依存性副作用の出現を予防する[1]．

2．CyAとTac薬物動態[2]の個人差

【CyAとTacの吸収・代謝・排泄】

● CyAは主に小腸で吸収され，その健常人でのbioavailabilityは平均で30％(5～70％)と報告されている．CyAの小腸での吸収にかかわる因子として腸管の長さ(腸管輸送時間)，胆汁流量，食事摂取(脂肪摂取・それによる胆汁流量変化，消化管運動)が挙げられる．胆汁は乳化剤として働き，その吸収を促進する．

● Tacも同様に小腸で吸収され，健常人，臓器移植後の患者でのbioavailabilityは平均で17～25％と報告されている．CyAと異なり，その吸収は胆汁流量に影響されないとされている．

● CyA，Tacともにチトクローム P-450(CYP)3A4によって主に代謝される．主な代謝部位は肝臓と吸収部位である小腸粘膜である．また，CYP3A4の活性に顕著な個人差・病態による差が認められる．このため肝障害を有する場合には肝での代謝が遅延する．

●肝臓・小腸粘膜で代謝されたCyA，Tacはともに P-糖蛋白質(未変化体・代謝物の担体)を介してそれぞれ胆汁中・小腸管腔内へ排泄される．P-糖蛋白質の発現にも明らかな個人差が認められる．胆汁流出障害・腸管機能異常(下痢など)によりクリアランスは変化する．

正常と異常の判断

1．CyA，Tacの用法と用量

前述したように，CyA，Tacに関するbioavailability，代謝に個人差があるため，各疾患・病態別に用法として初期投与量が設定されていても，その後は血中濃度を測定し，有効至適濃度を維持できるように投与量を調節する必要がある．移植術直後，CyA，Tacの十分な血中濃度を確保するために持続静注による投与が選択されるが，内服可能時には原則として朝・夕12時間間隔の経口投与に切り替えられる．

2．CyA，TacのTDM

1）検体

CyA，Tacはともに血液中では血球分画に高濃度に分布する．このため，血漿分離条件などが測定値に及ぼす影響を考慮して，全血をサンプル(抗凝固剤：EDTA-2K)とした測定が行われる．

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2）測定法

● CyA の測定法：高速液体クロマトグラフィ(HPLC)法，ラジオイムノアッセイ(RIA)法，蛍光偏光免疫測定(FPIA)法，酵素多量免疫測定(EMIT)法，ホモジニアス酵素免疫測定(CEDIA)法，ACMIA 法，このうち HPLC 法は研究目的が主であり，RIA 法は外注依頼が一般的である．FPIA 法は一般施設で最も汎用されている方法である．

● Tac の測定法：HPLC 法，酵素免疫抗体(ELISA)法，微粒子酵素免疫測定(MEIA)法が行われる．ELISA 法は外注検査機関で主に用いられており，感度が高く，低濃度サンプルでも正確な濃度測定が可能である．一方，MEIA 法は感度が劣るものの，迅速な測定が可能であり汎用されている．

3）TDM の実際

臓器移植時には過量投与による副作用の発現と低用量投与による拒絶反応の発現を防ぐために，移植直後には血中濃度の測定を頻回に行い，安定期には1か月に1回を目安に測定を行う．また，投与量を変更する場合，あるいは後述する相互作用のある薬剤の併用開始・中止の場合には必要に応じ頻回に測定する必要がある．

（1）トラフ値(次回投与前の最低血中濃度：CO)によるモニタリング

● CyA，Tac の薬物動態は種々の影響下にあり，食事による吸収の影響，後述する併用薬剤との相互作用による影響を少なくする点において，朝空腹時，併用薬剤内服前のトラフ値測定は優れていると考えられる．

●臓器移植においては，目的とする有効至適濃度域は移植臓器の種類・術後経過日数・移植施行施設によって異なる．

●一般に臓器移植後は移植後早期，特に1週～1カ月は拒絶反応の好発時期にあたり，この間は強力な免疫抑制(血中濃度を高く維持)を行い，それ以後徐々に減量する．

●一般に CyA で十分な免疫抑制を必要とする際には，目標とするトラフ値は 200～300 ng/m*l* であり，一般的な安全な治療レベルは 100～200 ng/m*l* とされる．臓器移植後はおおよそ経過を通して 100～400 ng/m*l* に設定されている．

● Tac の血中濃度は 20 ng/m*l* 以上が中毒域に近い濃度とされ，臓器移植後では術後1カ月まで血中濃度を 10～15 ng/m*l*，以後減量し 5～10 ng/m*l*，6カ月以上の長期には 5 ng/m*l* 前後で維持する施設が多い．

（2）服薬後2時間値(C2)モニタリング[3]

● CyA のマイクロエマルジョン製剤であるネオーラルの開発により，個体内における吸収のばらつきが改善され，投与後の血中濃度に再現性が得られるようになったことを契機に検討されたより精度の高い CyA 濃度のモニタリング法である．

● CyA の C2 モニタリングはトラフ値モニタリングに比べ効果と安全性の指標としての精度が高く，より正確な投与量調節が可能である．心移植・腎移植・肝移植においてはこのモニタリングで拒絶反応と腎障害が有意に減少することが明らかとなっている．

●脳死肝移植後における CyA の C2 値として，術後6カ月まで 1,000 ng/m*l*，6～12 カ月まで 800 ng/m*l*，12 カ月以降 600 ng/m*l*，腎移植においては6カ月まで 1,700 ng/m*l*，6～12 カ月まで 1,200 ng/m*l* が推奨されている．

異常を示す疾患・病態(薬物との相互作用)

CyA，Tac は，さまざまな薬物との相互作用をきたし，この薬物相互作用の機序は以下のように分類される．

（1）CYP3A4(主に肝臓・小腸)で代謝される他の薬剤が併用された場合，CyA，Tac の代謝が拮抗・阻害され，この2剤の血中濃度が上昇する．

（2）併用薬剤が小腸における CYP3A4 を直接阻害し血中濃度が上昇する．

（3）CYP3A4 を誘導する薬剤との併用により代謝が亢進し血中濃度が低下する．

（1），（2）では，同時に未変化体・代謝物の担体である P-糖蛋白質も阻害される場合があり，

表1　シクロスポリンとタクロリムスの血中濃度に影響を及ぼす薬剤

1）同一のCYP3A4で代謝されるため，代謝拮抗にて血中濃度が上昇
- Ca拮抗剤：ニフェジピン，ニルバジピン，ジルチアゼム，ニカルジピン
- 抗真菌剤：クロトリマゾール，フルコナゾール，ケトコナゾール，イトラコナゾール
- 抗生物質：エリスロマイシン，ジョサマイシン，クラリスロマイシン
- HIVプロテアーゼ阻害剤：リトナビル，サキナビル，ネルフィナビル
- その他：ブロモクリプチン，ダナゾール，シメチジン，オメプラゾール

2）小腸粘膜のCYP3A4とP-糖蛋白質を直接阻害し血中濃度が上昇
- グレープフルーツジュース，スイーティー，ザボン

3）CYP3A4が誘導され，代謝が促進され血中濃度が低下
- 抗てんかん剤：カルマバゼピン，フェノバルビタール，フェニトイン

4）CYP3A4とP-糖蛋白質が誘導され血中濃度が低下
- リファンピシン
- 西洋オトギリ草(セント・ジョーンズ・ワート)含有食品

排出が抑制されることにより血中濃度の上昇が増長される．同様に(3)の作用を有する薬剤の中にはP-糖蛋白質の消化管での発現を増加させるものがあり，この場合には排出の亢進により血中濃度の低下が促進される．表1に薬物相互作用の機序別にCyA，Tacと薬物相互作用をきたす薬剤を示す．

関連検査

CyA，Tacの副作用である腎機能障害・高K血症に関して定期的な検査が必要である．

検査費用と保険請求

CyA，TacのTDMに対しては特定薬剤治療管理料が1カ月に1回算定できる．臓器移植の場合には，移植月を含め3カ月間は3,210点/月，4カ月目より470点/月であり，Behçet病などの自己免疫疾患の場合では初月に750点/月，2カ月目より470点/月となる．

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