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CASE REPORT

Repeated intestinal perforation caused by an incomplete form of Behçet's syndrome

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Abstract: Behçet's disease, as initially described, is a triad of recurrent oral and genital ulcers and relapsing uveitis. The incomplete form, in which there is no ocular involvement, has been described in Japan and Korea, but this is not commonly recognized in the southern Chinese. We reported herein a rare case of repeated intestinal perforations caused by an incomplete form of Behçet's syndrome in a southern Chinese man.

Key words: Behçet's syndrome, Chinese, enterocolitis, intestine, perforation.

Introduction

Behçet's disease is prevalent in countries along the Silk route; Japan, Korea, northern China, and the Mediterranean.[1] The typical triad of recurrent oral and genital ulcers and relapsing uveitis is easily recognized.[2] The incomplete form, in which there is no ocular involvement, has been well described in Japan, but is rarely recognized in Chinese patients of southern origin.[3] We reported herein a rare case of repeated intestinal perforation caused by an incomplete form of Behçet's syndrome in a Chinese gentleman.

Case report

A 72-year-old Chinese man was admitted to hospital (Ruttonjee Hospital) for fever (40–76°C) and malaise in December 1996. He had a history of pulmonary tuberculosis 30 years ago and a pyloroplasty for pyloric stenosis caused by duodenal ulcers 10 years ago. In October 1996, he presented with 3-week history of a painful oral ulcer (1 cm) and fever after returning from Malaysia. Laboratory tests were negative for antinuclear factor, anti-double stranded DNA, rheumatoid factors and malaria. A serologic study revealed no detectable antibodies against cytomegalovirus, toxoplasma, HIV I and II and mycoplasma. Viral cultures were negative for Dengue fever, influenza, adenovirus, and Japanese B virus. Blood cultures grew no organisms. A chest radiography and abdominal sonography revealed no abnormalities. With a recent history of a visit to an endemic area with malaria, methoquine was prescribed, but his fever persisted. His fever and oral ulcers responded to a one week course of dexamethasone. He had no family history of significant medical illness. The origin of both his parents were of Guongdong province (Chung Shan) in southern China.

During his second admission in December 1996 for fever, a physical examination was unremarkable except for anemia. The laboratory investigation revealed a mild macrocytic anemia (hemoglobin 10.3 g/dL, mean cell volume 103 fl), leukocytosis (white blood cells 16 $\leftrightarrow 10^9$/L, neutrophils 12.3 $\leftrightarrow 10^9$/L), high erythrocytes sedimentation rate (93 mm in 1 h), normal serum vitamin B12, and a red cell folate level. A fecal hemoccult test was positive. Upper endoscopy demonstrated antral erosions and a histological examination demonstrated *Helicobacter pylori*-associated active chronic gastritis. The fever subsided after intravenous cefuroxime.

In February 1997, he was admitted again for a one week history of right lower abdominal pain, bloody diarrhea and fever (39°C). A blood test revealed leukocytosis of 20 $\leftrightarrow 10^9$/L. Cultures of feces and blood grew no organisms. A microscopic examination of feces for parasite were also negative. The anti-amoebic anti-

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bodies, Widal test and cANCA were negative. An abdominal radiography revealed free gas under the diaphragm. A laparotomy revealed several perforations arizing from discrete 0.5 ↔ 1.0 cm oval punched-out ulcers in the terminal ileum. Additional non-perforated ulcers were also found in the ascending and transverse colon. Twenty centimeters of terminal ileum and 53 cm of right-sided colon were resected. Histological examination revealed deep ulcers with predominant neutrophil infiltration. The adjacent mucosa was relatively normal (Fig. 1). A special stain revealed no cytomegalovirus inclusion bodies and amoeba. The Ziehl–Neelsen staining was negative for acid-fast bacilli.

In March 1997, he was readmitted for hematemesis. An upper endoscopy revealed several oesophageal ulcers of 1 cm in diameter and duodenal erosions. Antral biopsies again demonstrated *Helicobacter pylori*-related active chronic gastritis. Biopsies of the esophagus revealed non-specific ulcers. He was treated with omeprazole and anti-*Helicobacter* therapy (1 week course of tripotassium dicitrato bismuthate 120 mg fourfold daily, amoxicillin 500 mg thrice daily and metronidazole 400 mg thrice daily), and the symptoms subsided. In October and December 1997, he had two further episodes of fever and diarrhoea that responded promptly after a 1 week course of intravenous cefuroxime.

In January 1998, he presented again with fever, hematemesis, diarrhoea and abdominal pain. This time, multiple 1-cm painful superficial ulcers were present on the tongue, buccal mucosa and the scrotum. There was no uveitis or joint tenderness. Both sacro-iliac joints were normal radiologically. The pathergy test was negative. An upper endoscopy revealed erosive oesophagitis and antral erosive gastritis. Colonoscopy demonstrated multiple skipped 0.5 cm ulcers at the enterocolonic anastomosis. The intervening mucosa was intact (Fig. 2). He was treated with multiple antibiotics including cefuroxime, gentamicin, sulperazone and meropenem, but the fever and diarrhoea did not responded. Free gas in the abdomen was evident 4 weeks later. A second laparotomy demonstrated multiple 1-cm ulcerations with perforation at the ileo-colonic anastomosis, and resection of the perforated bowel was performed. A histological examination demonstrated non-specific ulcerations with predominant neutrophil infiltraton.

In August 1998, he presented with peritonitis again with free gas under the diaphragm. A third laparotomy demonstrated marked inflammation and ulceration around the ileum and anastomotic site. The intervening intestinal mucosa appeared normal. A pathologic examination of the resected bowel revealed fibromuscular intimal thickening of small arteries with > 50% occlusion of their lumen. Atherosclerosis or thrombosis was not observed (Fig. 3). In September 1998, he developed another attack of abdominal pain, diarrhoea and fever. There was recurrence of oral and scrotal ulcerations. He developed pneumoperitoneum with peritonitis (Fig. 4), but strongly refused an operation. Fortunately, his fever and abdominal pain subsided with sulperazone therapy. Thereafter, prednisolone (50 mg daily) was prescribed. After 1 month, the diarrhoea improved and the dose of prednisolone was gradually reduced to 35 mg/day in November 1998, and mesalazine (250 mg twice daily) was added. Unfortunately, 2 weeks after the reduction of prednisolone, he developed peritonitis. The fourth laparotomy revealed leakage at the ileo-colonic anastomosis, resulting in a large intra-abdominal abscess. A resection of the perforated bowel and ileostomy was performed. He finally died of septicemia.

Discussion

Our patient was suffering from recurrent enterocolitis with four episodes of bowel perforations within 2 years. The ulcerations were mainly located at the terminal ileum, proximal colon and around the ileo-colonic anastomosis after bowel resection. There was rectal sparing. A pathologic examination demonstrated marked neutrophil infiltration without crypt distortion, granuloma formation and vasculitis. Although the location of the ulcerations was typical of those found in Crohn's disease, the microscopic features of Crohn's disease, that is, chronic inflammation involving all layers of intestinal wall or granuloma, were absent. The classical features of Behçet's syndrome were recurrent oral and genital ulceration and uveitis.[4,5] This patient had no ocular involvement, but can be classified as an incomplete form of Behçet's syndrome, according to Morita *et al.*[6] This incomplete form of Behçet's colitis without ocular involvement had been well described in the Japanese.[3] In a recent epidemiologic study, this incomplete form was found in 38% of the Behçet's disease in Korea.[7] The pathergy test, which was positive in 90% of the Japanese or Turkish patients with Behçet's disease,[8] was negative in our patient. However, this could not exclude the diagnosis of Behçet's disease because the pathergy test was negative in 38% of the Chinese patients.[9]

It is interesting that his fever and abdominal pain was relieved by the use of antibiotic therapy on several occasions. This happened even when he had high fever and pneumoperitoneum. As there were no evidence that antibiotic therapy could reduce the activity of Behçet's enterocolitis, it is possible that spontaneous closure of the intestinal microperforation prevented the translocation of bacteria accounts for the 'success' of antibiotic therapy.

The pattern of organ involvement in Behçet's disease was different in Oriental and Meditarranean patients. Gastrointestinal involvement was uncommon in the Turkish patients, with an incidence ranging from

Figure 1 Deep undermined, punched-out ulcer at the ileocecal junction. The immediate adjacent mucosa appears to be relatively normal (HE stain, ↔40).

0 to 5%.[10,11] In contrast, the frequency of gastrointestinal involvement in Behçet's disease was higher in the Chinese (15%),[12] and was even higher in the Japanese (50–60%).[13,14]

The ulcerations in our patient were mainly located at the terminal ileum, proximal colon and the esophagus with a discontinuous distribution. The location of ulcers in the colon was consistent with previous reports[15,16] that the ulcers were mostly found in the terminal ileum and the cecum (75% of patients). However, rectal sparing, which had been observed in our patient, was seen in only 3% of patients with gastrointestinal Behçet's disease as reported by Plotkin.[15] The morphologic characteristics of the intestinal ulcer in our patient was also typical of Behçet's enterocolitis, that is, the ulcers were discrete with a punched-out appearance.[17] Discontinuous intestinal involvement was also a typical feature.[3] Oesophageal involvement, a feature in our patient, had also been documented, but was infrequent.[18,19]

The histological feature of enterocolitis in this patient was a non-specific acute inflammation with predominant neutrophil infiltration, resulting in small artery obliteration as shown in Fig. 3. This is a typical histological finding in Behçet's disease and was particularly common in the Asian patients.[18,20] The exact mechanism for the intense neutrophil infiltration was unknown. Sahin *et al.* found that these neutrophils had an exceedingly high adherence to the endothelial cells in the *in vitro* cell culture study.[21] This adhesion was further stimulated by inflammatory mediators such as interleukin-1, tumor necrosis factor and lipopolysaccharide.[21] The other less common pathologic feature of Behçet's enterocolitis not found in our patient was the chronic inflammatory change with transmural fibrosis and granuloma formation, which simulates Crohn's disease.[18,20]

Our patient suffered from four episodes of perforation at the ileum and proximal colon, which required surgical resection. Kasahara *et al.* reported that perforation of intestinal ulcers was common in Behçet's syndrome, but was distinctly unusual in Crohn's disease because of its intense fibrosis.[18] Furthermore, it was well known that surgical resection alone was associated with frequent recurrence of intestinal ulcers (46–77%) and further perforation.[22–24] Other rare causes of recurrent intestinal perforations are intestinal lymphoma,[25] eosi-

Figure 2 Colonoscopy revealed two oval ulcers (0.5 cm) at the ileum with normal intervening mucosa.

nophilic gastroenteritis,[26] Ehlers–Danlos syndrome,[27] and intestinal neurofibromatosis,[28] but these were not evident in our patient. The treatment of Behçet's enterocolitis was rarely reported. Only seven cases treated with steroids[29–35] have been reported in the English literature in the past 30 years. All patients, except for two had an excellent response to steroid therapy. The two patients who failed to respond to steroid therapy responded to thalidomide.[34,35] The poor outcome in our patient might be because of the late treatment or a too early reduction of corticosteroid.

In conclusion, Behçet's disease should become a differential diagnosis in patients with recurrent oro-genital ulcerations and enterocolitis with a non-specific histological feature, and that systemic corticosteroids or thalidomide treatment should be started early.

Key points

1. Behçet's disease, as initially described, is a triad of recurrent oral and genital ulcers and relapsing uveitis.
2. The incomplete form, in which there is no ocular involvement, has been described in Japan and Korea, but this is not commonly recognized in the southern Chinese.
3. We reported herein a rare case of repeated intestinal perforations caused by an incomplete form of Behçet's syndrome in a southern Chinese man.
4. In conclusion, Behçet's disease should become a differential diagnosis in patients with recurrent oro-genital ulcerations and enterocolitis with a non-specific histological feature, and that systemic corticosteroids or thalidomide treatment should be started early.

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EDITORIAL

Cerebral vascular resistance in hepatic insufficiency

Cerebral blood flow (CBF) in patients with cirrhosis and hepatic encephalopathy is generally thought to be decreased.[1–7] Decreased CBF has also been noted in patients with acute liver failure,[7] cirrhotic patients with subclinical encephalopathy,[8] and in those with alcoholic liver dysfunction.[9] In these studies, CBF was measured by using either the nitrous oxide method[1,2] or the xenon-133 inhalation technique.[3–9] The measured flow reduction has been between 16 and 42%; usually the worse the encephalopathy, the lower the CBF.[1,2]

The reason for the decrease in CBF is still unclear. It has been shown in experimental acute liver failure that arterial hypotension results in a fall in CBF because of failure in cerebral autoregulation.[10] Cerebral autoregulation induces reactive dilatation or constriction of cerebral resistance vessels, allowing CBF to remain virtually constant despite changes in the perfusion pressure.[11–13]

Patients with cirrhosis have a hyperdynamic circulation[14–16] with increased cardiac output and reduced peripheral vascular resistance, which often leads to arterial hypotension. A reduced metabolic rate for oxygen has been demonstrated in hepatic encephalopathy;[17] it may also contribute to decreased CBF.

A recent study using [^{99m}Tc]-hexamethylpropyleneamineoxime (HM-PAO) single-photon emission computed tomography (SPECT) (Nihon Mediphysics, Nishinomiya, Japan) imaging has shown a significant regional CBF reduction, ranging from 6 to 7% in the cortical region in the majority of the group of patients with cirrhosis as compared to controls.[18] This regional CBF reduction is normalized after liver transplantation. This evidence suggests a strong correlation between decreased CBF and severe liver damage.

The use of the Doppler method to measure blood flow velocity in intracranial arteries is a relatively new approach.[19–21] Although a transcranial Doppler (TCD) ultrasound measurement of blood flow velocities in the middle cerebral artery correlates with regional blood flow to some extent in healthy subjects,[22] absolute flow velocity obtained by TCD poorly correlates in symptomatic diseased patients.[23] This discrepancy and a large variability indicate that blood flow velocity measured by using TCD is not a reliable index of CBF.[23] In fact, attempts to measure flow velocity by TCD in patients with cirrhosis have yielded inconsistent values.[24–26]

Dillon *et al.* have reported that the mean cerebral blood velocity is decreased in advanced cirrhotic patients compared with normal controls.[24] When the findings of Lagi *et al.*[25] are compared with those of Larsen *et al.*,[26] no differences are noted in the mean cerebral blood velocity between cirrhotic patients and healthy controls. Moreover, in this issue of the *Journal of Gastroenterology and Hepatology*, Kawakami *et al.* also report that they could not demonstrate significant differences in the mean blood velocity of the middle cerebral artery between the control subjects and patients with liver cirrhosis.[27]

These data suggest that the mean cerebral blood velocity is not a reliable parameter for detecting CBF alterations, partly because of the poor reproducibility of the measurement of cerebral blood velocity. This is likely to be because of the fact that the required correction of the insonation angle.[26]

Pulsatility and resistance indices (PI and RI) are calculated from the waveform of the cerebral blood flow of the middle cerebral artery by the use of TCD.[26] In contrast to the blood flow velocity, pulsatility and resistive indices, which are widely accepted as indices of vascular resistance,[28] can be obtained without correction to the insonation angle, yielding a technically more reliable index of CBF with better reproducibility than for cerebral blood velocity.

According to Guevara *et al.*, the cerebral resistive index determined by the use of TCD in the middle cerebral artery is significantly increased in patients with ascites when compared with either ascite-free patients or healthy controls.[29] They assumed that cerebral vasoconstriction, which is probably related to arterial hypotension and overactivity of the vasoconstrictor systems, prevailed in patients with cirrhosis and ascites.

Similarly, Kawakami *et al.* have demonstrated that the cerebral vascular resistance indices (PI and RI) measured by TCD are increased in association with the severity of cirrhosis and hepatic encephalopathy.[29] Furthermore, according to their findings, the blood ammonia and serum albumin levels influence the change of cerebral resistance in cirrhotic patients.

A marked elevation in cerebral vascular resistance at the decompensated cirrhotic stage may be caused by an overactivation of the renin angiotensin aldosterone system and the sympathetic nerve system secondary to increasing systemic arterial vasodilatation.[30–32] In addition, patients with advanced cirrhosis often exhibit impaired CBF autoregulation.[10,25,28,33] Therefore, in pa-

tients with advanced cirrhosis and high cerebral resistance, systemic hypotension may result in cerebral hypoperfusion under impaired CBF autoregulation.

Kawakami *et al.* have demonstrated that hepatic encephalopathy is clearly related to increased cerebral vascular resistance, which may be altered by the severity of hepatic encephalopathy in each individual.[29] The mechanism by which cerebral vascular resistance is increased in hepatic encephalopathy has not been elucidated. In other words, it is not known whether hepatic encephalopathy causes increased cerebral vascular resistance, or vice versa.

In contrast to the above-mentioned theory, the cerebral autoregulation system might increase the cerebral vascular resistance as a result of exacerbation in the severity of liver diseases, even in hepatic encephalopathy. The combined intracranial volume of blood, cerebrospinal fluid and the brain mass remain unchanged to avoid a rise in intracranial pressure. A primary rise in brain volume caused by an edema leads to a decompensated state in which even a slight increase in cerebral blood volume results in a drastic rise in intracranial pressure. It has been assumed that even a slight CBF increase enhances cerebral blood volume. According to the hypothesis of gradual cerebral hyperemia, cerebral autoregulation may gradually increase cerebral vascular resistance in the early course of hepatic dysfunction or encephalopathy. At the later or terminal stage of hepatic insufficiency such as hepatic coma, cerebral autoregulation may gradually be neutralized to subsequently influence the volume-pressure relationship, producing brain edema.[37]

No matter how cerebral vascular resistance is regulated, or whether it is a chicken or an egg situation, the evidence that cerebral vascular resistance is increased in patients with severe cirrhosis, especially in patients with hepatic encephalopathy, is very important. As such, cerebral vascular resistance measured by using the TCD method may provide a reliable index for monitoring severely affected patients with cirrhosis with or without hepatic encephalopathy.

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LETTER TO THE EDITOR

A short period of interferon therapy led to a sustained response in two cases of chronic hepatitis C

Interferon (IFN) is the only evidence-based antiviral treatment widely used for chronic hepatitis C (CHC).[1,2] In Japan, the most popular regimen is a high-dose IFN therapy for 24 weeks, composed of daily and intermittent administration, regardless of the patient's background. However, we experienced two cases with CHC who have been led to a sustained response by a very short course of IFN therapy.

The first case was a 36-year-old man who was admitted to our hospital (University of Occupational and Environmental Health Hospital) for IFN treatment for CHC on 14 May 1997. The genotype of HCV was 2a as detected by the use of the RT-PCR method, and the quantity of HCV-RNA in serum was 4.0 kcopies/mL as detected by the Amplicor-HCV monitor assay, and less than 0.5 Meq/mL by using the branched DNA probe assay. Liver histology revealed chronic hepatitis (CH) with moderate inflammation and mild fibrosis in the portal area. On May 26 1997, IFN therapy was started under a schedule of daily administration of IFN a-2b (Intron A, Schering-Plough, Kenilworth, NJ, USA) at a dose of 10 million units (MU) for the initial 2 weeks and threefold a week for the following 22 weeks. However, IFN therapy was discontinued on June 16 because the patient had severe general fatigue. The total dose of IFN that the patient received during the 3 weeks of therapy was only 146 MU. At the time IFN therapy was stopped, serum aminotransferase levels were normal and HCV-RNA was not detectable by qualitative analysis using Amplicor-HCV kits (Nippon K.K., Tokyo, Japan). Both normal aminotransferase levels and the negative of serum HCV-RNA were sustained without any medication for 18 months after IFN therapy ended.

The second case was a 35-year-old woman who was admitted to our hospital for the purpose of IFN therapy for CHC on 20 January 1999. The quantity of serum HCV-RNA was 138.0 kcopies/mL as detected by using the Amplicor-HCV monitor assay, and less than 0.5 mL when the branched DNA probe assay was used. The hepatitis C virus genotype was 2b as detected by the use of RT-PCR. Histological findings of the liver showed CH with mild activity and minimal portal fibrosis. Interferon therapy with IFN a-2b was started on 8 February at a daily dose of 6 MU for the initial 6 days, and was thereafter increased to 10 MU. Interferon therapy, however, was discontinued on 26 February because of severe neutropenia. The total dose of IFN was only 91 MU. When the IFN therapy ended, HCV-RNA was not detectable in serum by the use of qualitative analysis with an Amplicor-HCV kit, and aminotransferase levels were normal. The neutropenia improved rapidly. In this patient, HCV-RNA was negative and aminotransferase levels are normal without any medications even 6 months after the final injection of IFN (Fig. 1).

Although IFN is an antiviral treatment widely used for chronic hepatitis C, many patients experience a relapse after IFN therapy is stopped. Many randomized control studies on IFN therapy for CHC have suggested that a longer therapeutic period and/or a higher dose of IFN is necessary to prevent a relapse.[1–4] Moreover, the disappearance of HCV-RNA in serum during the administration of IFN does not assure the cure of CHC. In Japan, therefore, a high-dosage of IFN therapy for 6 months has been uniformly adopted for many patients with CHC, if they do not have any complications. A high-dosage of IFN therapy for 6 months was initially made a plan in our cases. However, because severe general fatigue (case 1) or neutropenia (case 2) occurred after the start of the treatment, IFN administration was discontinued in each case after 3 weeks.

Interferon therapy for patients with a low viral load has been indicated to be more effective than it is for those with a high viral load. Patients infected with HCV genotype 2a or 2b are more likely to respond to IFN therapy than those infected with HCV genotype 1b.[2,3] Both of the present cases were aged under 40 years, and their liver fibrosis was mild. Moreover, the HCV genotypes of these two patients were 2a and 2b, respectively, and the quantity of HCV-RNA was low in both cases. These data indicate that both of the patients were IFN sensitive. It was considered, therefore, that they became sustained responders despite the short course with the low total dose of IFN. These cases suggest that the duration of IFN therapy can be shortened, and that the total dose of IFN can be reduced for IFN-sensitive patients. If the same sustained response rate is expected, a short schedule of IFN therapy is much better than the Japanese usual schedule for IFN therapy, which requires IFN administration for 6 months and is sometimes accompanied by serious adverse effects.[5] However, the optimal dose of IFN and the adequate schedule for sensitive subjects remains unsolved.

In future studies, it will be necessary to determine the adequate regimen of IFN therapy, based on the patient's backgrounds.

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ORIGINAL ARTICLE

Characterization of hepatitis B virus surface antigen-specific CD4+ T cells in hepatitis B vaccine non-responders

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Abstract

Aim: To study the mechanisms of hepatitis B vaccine non-response, we examined hepatitis B virus surface antigen (HBsAg)-induced proliferation of peripheral blood mononuclear cells (PBMC) obtained from hepatitis B (HB) vaccinees.

Methods: Subsequently, we have examined the features of HBsAg-reactive CD4+ T cells in HB vaccine non-responders (NR). Based on serum anti-HBs titers, we divided these vaccinees into three groups: high responder (HR), middle responder (MR) and non-responder (NR), and examined HBsAg-induced proliferation of their PBMC.

Results: We found that the *in vitro* response of PBMC to stimulation with HBsAg was correlated with their serum anti-HBs titer (mean stimulation index was 10.71 in HR, 4.37 in MR and 1.96 in NR). However, by the deletion of CD8+ T cells, the increased response was observed in two of four NRs.

Conclusions: The present results have also shown that at least four distinct HBsAg-reactive CD4+ clones existed (Vb17 + clone restricted with DR4, Vb8 + clone restricted with DQ7, and both Vb5.1 + clone and Vb20 + clone restricted with either DR9 or DQ3) in NRs. The results demonstrated that heterogeneous HBsAg-reactive CD4 clones existed in some HB vaccine NRs.

Key words: CD4 clones, hepatitis B vaccine, non-responder, T-cell receptor.

Introduction

Since the development of hepatitis B (HB) vaccine, its efficacy for protection against hepatitis B virus (HBV) infection has been well documented.[1] However, all previous studies have shown that 5–10% of HB vaccinees produced little or undetectable amounts of anti-HBs following a standard course of immunization.[2,3] The lack of immune response to foreign antigen has been shown to be mediated by suppressor T cells,[4,5] by the lack of antigen-presenting cells (APC),[6–9] and defective antigen-reactive T cells.[10–14] Extensive studies have established that the humoral immune response to hepatitis B surface antigen (HBsAg) was genetically controlled both in mouse[15] and human.[2,3] In contrast, several previous reports have indicated that HBsAg-reactive T cells were not defective in HB vaccine non-responders (NRs), but rather assumed that this non-responsiveness was mainly caused by the presence of antigen-reactive suppressor T cells.[17,18,20]

Therefore, in the present study, we attempted to determine whether HBsAg-specific CD4+ T cells were defective in HB vaccine NR.

Methods

Human subjects

We randomly selected HB vaccinees in this study from the vaccinated medical staff at our institute. All vaccinees examined in the present study were female doctors and medical students at our university, ranging in age from 23 to 36 years. We immunized these healthy volunteers with a recombinant HB vaccine (Bimmugen; Chemo-Sero Institute, Kumamoto, Japan)

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three times (0, 1 and 6 months) by using a standard protocol. The serum titer of anti-HBs was examined by using a commercially available kit (IMX, AUSAB; Abbott Lab., North Chicago, IL, USA), and the detection limit of this system was 2mIU/mL. Based on the serum anti-HBs titer at 7 days after the third immunization, these vaccinees were divided into three groups; high responder (HR), having a high anti-HBs, non-responder (NR) who produced undetectable amounts of anti-HBs, and the remainder designated as middle responders (MR). All vaccinees were then further immunized with 10 μg HB vaccine, and 1–2 weeks after this booster immunization, their peripheral blood mononuclear cells (PBMC) were examined in an *in vitro* study as described below. Among the seven NRs, five produced a small amount of anti-HBs after the fourth immunization, suggesting that these subjects were not real NRs, although their anti-HBs titers were relatively low (anti-HBs titer in four of them are shown in Table 1).

In vitro hepatitis B virus suface antigen-induced proliferation

To examine the *in vitro* response to the stimulation with HBsAg, PBMC were obtained from heparinized venous blood after centrifugation with a Ficol–Hypaque density gradient at 1–2 weeks after the fourth immunization. The culture medium was composed of TIL medium (Immuno-Biological Lab., Gunma, Japan) containing 2% human AB serum and antibiotics. Human AB serum used in the present study was selected from five healthy males to test its efficacy on PBMC response to HBsAg. Cells (1×10^5; either whole PBMC or CD8+ T cell-deleted cells; CD8-cells) were cultivated in 100 μL culture medium with or without 1 μg/mL HBsAg obtained from Chemo-sero Institute for 4 days. All cultures were set up in triplicate in 96-well U-bottomed culture plates (ICN Biomedicals, Aurora, CA, USA) and incubated in a 5% CO_2 containing atmosphere. In the culture was pulsed with 1 μCi [^{3}H]-Thy. The results were expressed at a stimulation index (SI).

The deletion of CD8+ T cells was carried out in three HR and four NR randomly selected vacinees by using the previously reported method, and using bead-conjugated antibodies.[22] Briefly, 5×10^6 PBMC were incubated with 10 μL anti-CD8 (PharMingen, San Diego, CA, USA) in 1 mL PBS at 4°C for 30 min. After washing the cells with PBS, they were further incubated with 2 mL PBS-BSA and 1 mL buffer, which contained antimouse immunoglobulin conjugated beads for 60 min at 4°C. The cells were then passed through a magnetic column to deplete the bead-conjugated cells. The purity of these negatively selected cells was analyzed by using a FACScan (BDIS Co., Mountain View, CA, USA) using FITC-conjugated anti-CD4 (PharMingen) or anti-CD8 antibody (PharMingen). Contamination of CD8+ T cells in these cell fractions was less than 2%.

Preparation of CD4+ T-cell clones

For preparation of the CD4 clones, 1×10^4 CD8-deleted cells were cultivated in 200 μL culture medium containing 1 μg/mL HBsAg in 96-well culture plates for 7 days. Subsequently, the cells were further stimulated with a condition medium composed of culture medium containing 100 IU/mL IL-2 (Shionogi Phar. Co., Osaka, Japan) and 1 μg/mL PHA (Sigma Co., St Louis, MO, USA). Approximately 14–21 days after the cultivation, the response of the cells in each well to HBsAg was examined, and functioning lines were selected (SI was > 3.0). The cloning of these lines was performed by using an ordinary limiting dilution method by seeding 0.5 cells/well in condition medium containing 1 μg/mL HBsAg. The clones were stimulated with HBsAg plus irradiated autologous PBMC as a APC source at a 2-week interval, and in the middle of this antigen stimulation, the condition medium was also changed. Their proliferative activity to HBsAg was examined by the incubation of 1×10^4 clones together with 1×10^5 irradiated autologous PBMC in 100 μL culture medium containing 1 μg/mL HBsAg for 4 days, and the assay was done as described above.

Analysis of T cell receptor Vb usage and MHC-restriction of clones

The features of these clones were examined by using the following two procedures. First, the use of T cell receptor *Vb* gene was analyzed by using fluoresceine isothiocyante (FITC)-conjugated antibodies to various TCR Vb molecules (T cell Product, Aichi, Japan). In brief, 1×10^4 cells of each clone were incubated with 5 μL of each FITC-conjugated antibody for 30min at 4°C. After washing, the positively stained cells were analyzed by using the FACScan. Second, to examine the MHC class II-restriction of each clone to exert their function, we obtained PBMC for MHC class II-identical or non-identical HB vaccinees. After these cells were irradiated with 3000 R, they were mixed with each clone and cultivated as described above.

Statistical analysis

Statistical analysis was done by using a Student's *t*-test. A *P* value less than 0.05 was considered significant.

Results

In vitro response of peripheral blood mononuclear cells

To examine the *in vitro* immune response to HBsAg, we used the selected human AB serum and adequate culture medium (as previously described) to test the ability to support the response to HBsAg. As shown in Fig. 1, PBMC obtained from five out six HRs responded well

Table 1 Goodman Hardie financial performance

Year	Net profit	Earnings per share
1997	A$8.5 m	10.5¢
1998	A$12.5 m	10.7¢
1999	A$15.4 m	10.2¢
2000	A$35.6 m	10.5¢

Source: GHP financial statements

to the stimulation with HBsAg (mean SI was 10.71). On the contrary, PBMC obtained from all NRs did not respond to the same stimulation of the HBsAg used (their mean SI was 1.96). The response of PBMC obtained from MR was between the responses for that of HR and NR (their mean SI was 4.37).

Deletion of CD8+ T cells from non-responders restores the response to HBsAg

To analyze the mechanisms of this *in vitro* non-response of NR's PBMC, we addressed the question of whether the depletion of CD8+ T cells from these cells prior to the *in vitro* stimulation would influence their response to the stimulation with HBsAg. We took PBMC from three HRs, as well as from four NRs after their deletion of CD8+ T cells (CD8-cells), and both whole PBMC and CD8-cells were then cultivated with HBsAg.

Probably because of the deletion of non-specific suppressor T cells, their cpm of HBsAg-cells were varied in each subjects. The results shown in Table 1 demonstrated that HBsAg-induced proliferative activity was equally observed in both whole PBMC and CD8-cells of the three HRs. Simultaneous studies showed that while the whole PBMC obtained from the four NRs did not respond to the stimulation with HBsAg, the deletion of T cells resorted the response to the stimulation with HBsAg in two of them, but not in the other two.

Preparation of HBsAg-reactive CD4+ clones

To support the results shown in Table 1, in which HBsAg-reactive CD4+ T cells also existed in NR, we attempted to establish HBsAg-reactive CD4+ T cell clones derived from two NRs (NR1 and NR2). As shown in Fig. 2, we were able to establish 11 CD4 reactive clones. All these clones responded to HBsAg (SI was > 5 in all clones). Their specificity was demonstrated by their lack of response to stimulation with HBcAg. Thus, these results indicate that HBsAg-reactive CD4 clones existed in HB vaccine NR, but they did not produce a detectable amount of anti-HBs after receiving a standard course of HB vaccine.

Use of T cell receptor Vb and MHC class II restriction

We further characterized these CD4 clones by studying their use of the TCR Vb molecule. Among the 11 clones, we successfully detected the expressed of the TCR Vb molecule on their cell surface of the six clones obtained only from NR1. Among these six clones, one expressed Vb17, one expressed Vb8, one expressed Vb5.1, and others expressed Vb20. A FACS pattern of one of them (clone E5–9) is illustrated in Fig. 3.

Based on the fact that their proliferative activity was inhibited by anti-MHC class II antibody (data not shown), we examined the precise restriction element of the four clones expressing distinct Vb molecule for exerting their function. As shown in Table 2, four clones expressing distinct TCR Vb molecules also revealed a distinct restriction for exerting their function. The B3–2 clone, expressing Vb17+, proliferated mainly by stimulation with HLA-DR4 identical allogenic APC, B3–5 clone, and Vb8+ (reacted only with DQ7-identical APC), and both the E5–9 clone, Vb5.1+, E5–10 clone, and Vb20+, reacted with DR9/DQ3-identical APC. Among them, B3–2 mainly reacted with DR4-identical APC, but also with DR9/DQ3-identical APC, and reverse results were obtained in the study of E5–9.

Discussion

The aim of the present study was to analyze the mechanism of HB vaccine non-responsiveness. Several previous reports have shown that, under certain conditions, PBMC obtained from HB vaccinees respond to HBsAg *in vitro*. However, many of those studies used both

Table 2 Goodman Hardie financial performance

Year	Net profit	Earnings per share	Cost per share	Net assets	Return on equity
1997	A$8.5 m	10.5¢	10.7¢	107¢	N/A
1998	A$12.5 m	10.7¢	10.7¢	111¢	10.5%
1999	A$15.4 m	10.2¢	10.7¢	122¢	5.7%
2000	A$35.6 m	10.5¢	10.7¢	121¢	8.7%

Source: GHP financial statements

HBsAg and certain T cell mitogens, such as PHA[17,18,20] and IL-2.[19] After examining several culture conditions, we found that the culture medium composed of TIL medium, originally prepared for maintaining T cell lines, and AB serum donated by a healthy man constantly provided HBsAg-induced proliferation. The usefulness of this *in vitro* system was demonstrated by the fact that the degree of the *in vitro* response to HBsAg was correlated with the serum anti-HBs titers (Fig. 1).

Using this *in vitro* culture system, we have shown that by the deletion of CD8+ T cells, two of four NR's PBMC showed a response to HBsAg (Table 1). Regarding the mechanisms of the immunological non-responsiveness to HB vaccine, several possibilities have been put forth, such as the defect of either HBsAg-reactive T cells[19] or antigen-presenting cells,[21] and the inhibitory effect of CD8+ T cells.[17,18,20,22] The results of the present study clearly demonstrated that the defect of HBsAg-proliferation of some of NRs was not because of the defect of either HBsAg-reactive T cells or APC, but rather because of the inhibitory effect of CD8+ T cells. These results were similar to those of the previous studies, including ours.[17,18,20]

To verify further the features of these HBsAg-reactive CD4+ T cells, we have attempted to establish CD4+ T cells clones derived from two NRs. As shown in Fig. 2, we were able to prepare 11 HBsAg-reactive CD4 T cell clones, indicating that the defect of HBsAg-reactive CD4 T cells in at least these two NRs was unlikely. The features of these CD4+ clones in NR were examined by their use of the TCR Vb molecule, as well as by their restriction element to exert their function. We found that at least four distinct TCR Vb molecules were used in these clones and that they exhibited a distinct MHC restriction manner to exert their proliferative activity (Table 2). Among these four distinct clones, B3–2 reacted mainly with APC, and E5–9 reacted mainly with DR9-identical APC. However, these two clones also reacted with other APCs (B3–2 also reacted with DQ3-identical APC, and E5–9 also reacted with DR4-identical APC, respectively). Because these two clones have expressed a single TCR Vb molecule, these phenomenon might be explained by the cross-reactivity rather than that these two clones contained several distinct clones.

The present results have indicated that as the previous results of CD4 clones obtained from HR's PBMC,[24,25] heterogeneous HBsAg-reactive CD4+ T cells also exist in NR. We must still use care in making such a conclusion because three of the four NRs analyzed here could make anti-HBs after the fourth immunization (Table 1), indicating that these subjects were not real non-responders, and thus should be designated as low responders. In addition, by using this *in vitro* culture system, we were not able to restore HBsAg-induced proliferative activity even after the deletion of CD8+ T cells in two of four NRs. Therefore, other possibilities such as the defect of either HBsAg-reactive CD4+ T cells or APC, perhaps existing in some of the HB vaccine non-responders, cannot be ruled out. In fact, Kruskall *et al.* have previously reported that such negative regulation by T cells of the immune response to HBsAg was not observed in HB vaccine NR.[18] In their study, however, to reveal the precise genetic factor, they analyzed a specific group of subjects having only HLA-B8, SC01, or DR3 phenotype. In addition, Chedid *et al.* have also reported that the precursor frequency of HBsAg-reactive T cells was remarkably low in NR subjects, and hence, they predicted that the lack of this *in vitro* response to HBsAg in PBMC of NR was because of the defect of CD4+ T cells.[23] The discrepancy between their results and the present study results might be because of the presence of CD8+ T cells that suppress the function of CD4+ T cells, or perhaps the subjects they examined may have had other regulatory mechanisms. Based on these previous studies.

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Key points

1 Behçet's disease, as initially described, is a triad of recurrent oral and genital ulcers and relapsing uveitis.
2 The incomplete form, in which there is no ocular involvement, has been described in Japan and Korea, but this is not commonly recognized in the southern Chinese.
3 We reported herein a rare case of repeated intestinal perforations caused by an incomplete form of Behçet's syndrome in a southern Chinese man.
4 In conclusion, Behçet's disease should become a differential diagnosis in patients with recurrent ulcerations.

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REGIONAL NEWS

Journal launched at Durban conference

Sample 3 col head

The reason for the decrease in CBF is still unclear. It has been shown in experimental acute liver failure that arterial hypotension results in a fall in CBF because of failure in cerebral autoregulation.[10] Cerebral autoregulation induces reactive dilatation or constriction of cerebral resistance vessels, allowing CBF to remain virtually constant despite changes in the perfusion pressure.[11–13]

Patients with cirrhosis have a hyperdynamic circulation[14–16] with increased cardiac output and reduced peripheral vascular resistance, which often leads to arterial hypotension. A reduced metabolic rate for oxygen has been demonstrated in hepatic encephalopathy;[17] it may also contribute to decreased CBF.

A recent study using hexamethylpropyleneamineoxime single-photon emission computed tomography (SPECT) (Nihon Mediphysics, Nishinomiya, Japan) imaging has shown a significant regional CBF reduction, ranging from 6 to 7% in the cortical region in the majority of the group of patients with cirrhosis as compared to controls.[18] This regional CBF reduction is normalized after liver transplantation. This evidence suggests a strong correlation between decreased CBF and severe liver damage.

The use of the Doppler method to measure blood flow velocity in intracranial arteries is a relatively approach.[19–21] Although a transcranial Doppler (TCD) ultrasound measurement of blood flow velocities in the middle cerebral artery correlates with regional blood flow to some extent in healthy subjects,[22] absolute flow velocity obtained by TCD poorly correlates in symptomatic diseased patients.[23] This discrepancy and a large variability indicate that blood flow velocity measured by using TCD is not a reliable index of CBF.[23] In fact, attempts to measure flow velocity by TCD in patients with cirrhosis have yielded inconsistent values.[24–26]

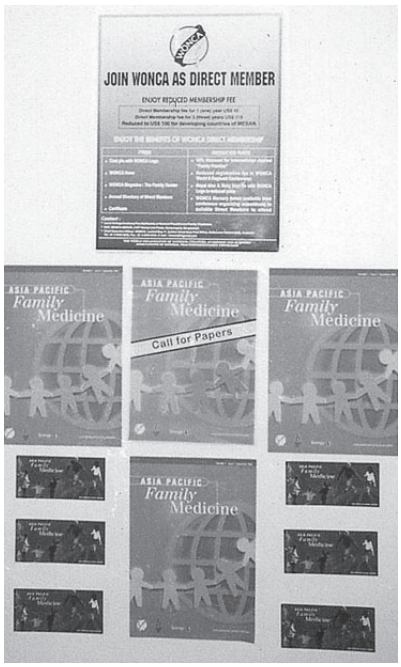
Dillon *et al.* have reported that the mean cerebral blood velocity is decreased in advanced cirrhotic patients compared with normal controls.[24] When the findings of Lagi *et al.*[25] are compared with those of Larsen *et al.*,[26] no differences are noted in the mean cerebral blood velocity between cirrhotic patients and controls. Moreover, in this issue of the *Journal of Gastroenterology and Hepatology*, Kawakami *et al.* also report that they could not demonstrate significant differences in the mean blood velocity of the middle cerebral artery between the control and patients with liver cirrhosis.[27]

These data suggest that the mean cerebral blood velocity is not a reliable parameter for detecting CBF alterations, partly because of the poor reproducibility of the measurement of cerebral blood velocity. This is likely to be because of the fact that the required correction of the insonation angle.[26]

Another head

The reason for the decrease in CBF is still unclear. It has been shown in experimental acute liver failure that arterial hypotension results in a fall in CBF because of failure in cerebral autoregulation.[10] Cerebral autoregulation induces reactive dilatation or constriction of cerebral resistance vessels, allowing CBF to remain virtually constant despite changes in the perfusion pressure.[11–13]

Pulsatility and resistance indices (PI and RI) are calculated from the waveform of the cerebral blood flow of the middle cerebral artery by the use of TCD.[26] In contrast to the blood flow velocity, pulsatility and resistive indices, which are widely accepted as indices of vascular resistance,[28] can be obtained without correction to the insonation angle, yielding a technically more reliable index of CBF with better reproducibility than for cerebral blood velocity.



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According to Guevara *et al.*, the cerebral resistive index determined by the use of TCD in the middle cerebral artery