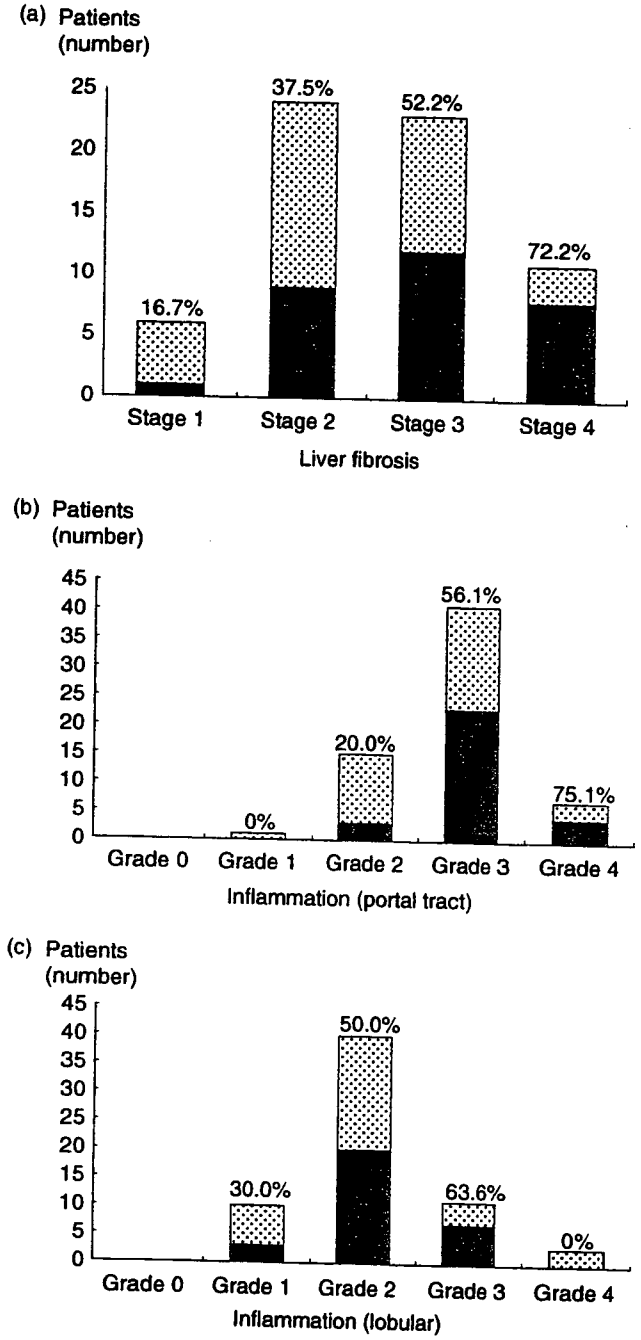


Figure 5 The fibrosis stage and inflammation grade in resected patients in relation to the frequency of oxyphilic granular hepatocyte (OGH). (a) A higher stage of fibrosis was associated with a higher frequency of OGH. (b) A higher grade of portal tract inflammation was associated with a higher frequency of OGH. (c) No relationship was found between the grade of lobular inflammation and the appearance of OGH. (⊡), OGH (−); (■), OGH (+).

Table 1 Fibrosis stage and inflammation grade (Ludwig classification) in relation to the frequency of OGH in the resected patients

Parameter	OGH	No OGH	*P*-value†
Stage			
1–2	10	20	0.041
3–4	20	14	
Grade (portal)			
0–2	3	13	0.009
3–4	27	21	
Grade (lobular)			
0–2	23	27	0.167
3–4	7	7	

†Chi-squared test, frequency of OGH in stage 1–2 *vs* 3–4 or grade 0–2 *vs* 3–4.
OGH, oxyphilic granular hepatocyte.

zone, but only slightly in the peripheral zone (Fig. 6). The mean mitochondrial score for the peripheral zone was 134.47 ± 27.24 (range 100–196) in the group with mild fibrosis, 156.82 ± 30.14 (range 107–227) in the group with severe fibrosis, and 128.89 ± 24.03 (range 100–193) in the control group. Thus, the score for the peripheral zone was significantly higher in the severe than in the mild fibrosis group. The amount of mitochondria in the peripheral zone tended to increase with the progression of fibrosis (Fig. 7).

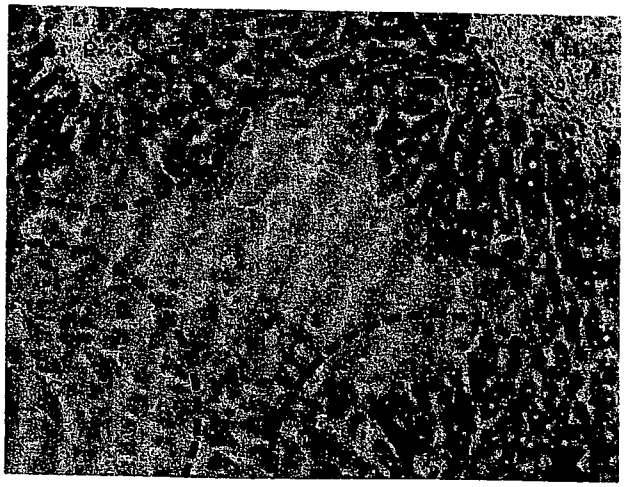


Figure 6 Immunohistochemical staining with antimitochondrial antibody. Hepatocytes with increased or decreased mitochondria were distributed in an irregular, map-like manner. The amount of mitochondria in hepatocytes was increased or maintained in the peripheral zone (P), but was markedly decreased in the central zone (C) in some patients. (Original magnification ×40.)

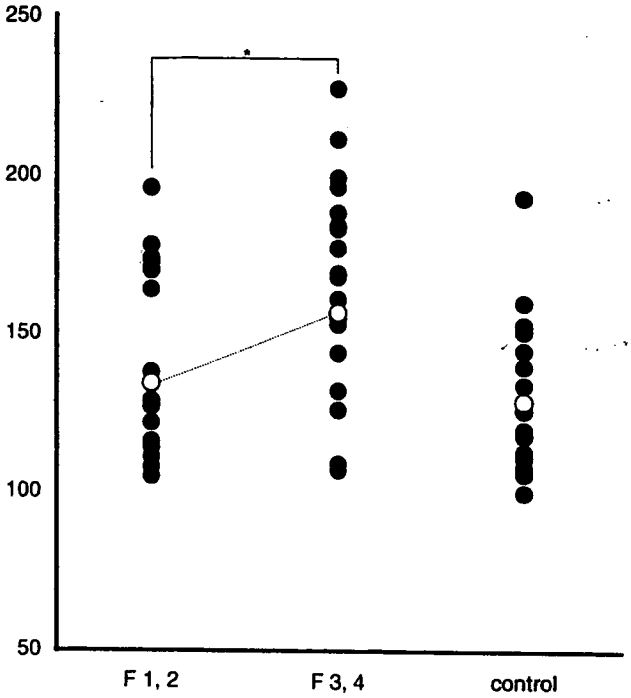


Figure 7 The relationship between the mitochondrial score and liver fibrosis in the peripheral zone. The mean mitochondrial scores for peripheral zone in the group with mild fibrosis, the group with severe fibrosis, and the control group were 134.47 ± 27.24 (100–196), 156.82 ± 30.14 (107–227), and 128.89 ± 24.03 (100–193), respectively. Thus, the mitochondrial score for peripheral zone was significantly higher in the group with severe fibrosis than in the group with mild fibrosis (*$P = 0.011$, Mann–Whitney U-test).

Hepatocyte mitochondrial function

Immunohistochemical staining with antimitochondrial antibody showed increased SDH and COX activity in the area with an increased amount of mitochondria (Fig. 8).

Changes in hepatocytes

There was increased expression of HXK-II in hepatocytes with increased mitochondria (Fig. 9), but not in control cases.

The mean Ki-67 labeling indices for the peripheral and central zones were 0.56 and 0.47, respectively, showing no significant differences among zones.

DISCUSSION

LEFKOWITCH *ET AL.* examined OGH in hepatitis B.[1] The frequencies of OGH in liver biopsy specimens in their study and ours were 41.7% and 35.5%, respectively, which appear to be roughly similar. They reported that OGH tended to appear in hepatitis B patients with severe fibrosis and inflammatory cell infiltration: among the liver biopsy specimens from their hepatitis B patients, the frequency of OGH was as high as 54.5%, whereas no OGH was observed in patients with nonactive cirrhosis. We determined the stage of fibrosis and grade of inflammation according to the Ludwig classification of chronic hepatitis.[6] In chronic hepatitis C, the frequency of OGH tended to be high in patients with severe fibrosis and portal tract inflammation (stage 3–4 or portal grade 3–4), and no OGH appeared in patients with mild fibrosis and portal tract inflammation (stage 1–2 or portal grade 1–2). Although it is difficult to accurately compare their results with ours, because of the difference in the method of classification of chronic hepatitis, we consider that the histopathological features of the background liver in which OGH appears are similar in hepatitis B and C. A similar tendency was observed in the resected patients.

No studies have examined the relationship between the appearance of OGH and the duration of hepatitis B. The present study showed that the duration of infection

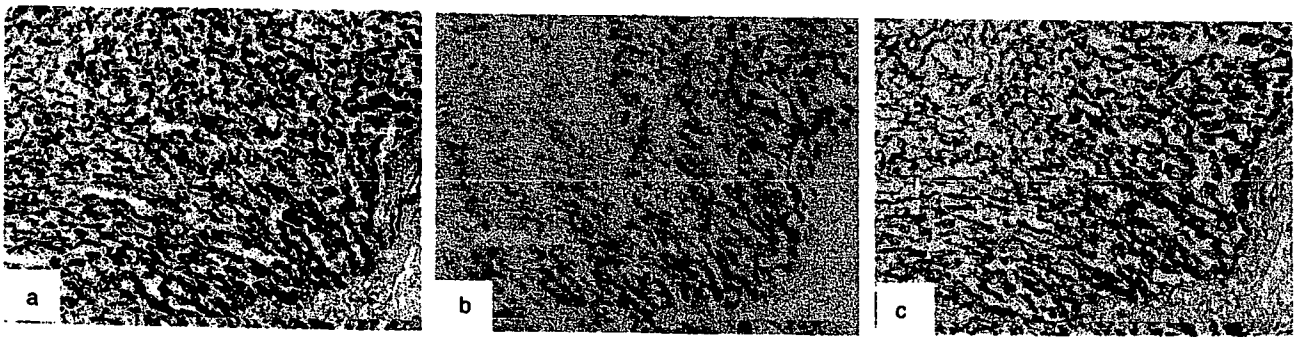


Figure 8 Serial frozen sections stained (a) with antimitochondrial antibody, (b) for succinate dehydrogenase (SDH), or (c) for cytochrome c oxidase (COX). SDH- and COX-positive cells were stained deep blue and brown, respectively. The expression of SDH and COX was increased in hepatocytes with increased mitochondria.

Figure 9 Immunohistochemical staining with (a) antimitochondrial antibody and (b) anti-hexokinase (HXK)-II antibody, showing HXK-II expression was increased in hepatocytes with increased mitochondria. (Original magnification ×40.)

in hepatitis C was closely related to the appearance of OGH: a longer duration of infection and a more advanced stage of fibrosis tended to be associated with a higher frequency of OGH. In patients with a long duration of infection, OGH appeared in the absence of cirrhosis. In contrast, in patients with an infection duration of less than 20 years, OGH did not appear in the presence of severe fibrosis. These findings suggest that the duration of infection is a factor in the development of OGH, which is related to long-term chronic infection. With little supporting evidence, we can only speculate that the persistence of chronic infection, hepatitis C virus infection of mitochondria, and oxidant stress for more than 20 years may alter mitochondria.

Immunohistochemical staining with antimitochondrial antibody allowed us to more objectively evaluate mitochondria in hepatocytes, and showed that mitochondria were increased not only in OGH, but also in other hepatocytes. Conversely, hepatocytes with markedly reduced mitochondria were clearly identified. An increase, but not a decrease, in the amount of hepatocyte mitochondria in chronic liver disease has been reported in several reports. Moreover, hepatocytes with increased or decreased mitochondria were distributed in an irregular, map-like manner. However, hepatocytes with increased mitochondria tended to be more frequently found in the peripheral zone, and those with decreased mitochondria in the central zone. It has been reported that chronic viral hepatitis is histologically characterized by the irregular distribution of liver cell necrosis and regeneration.[13–15] The irregular distribution of hepatocytes with increased or decreased mitochondria in this study may be related to the previously reported irregular distribution of lesions of chronic viral hepatitis.

Among the hepatitis C patients studied, OGH appeared in 47.1% of the liver biopsy patients and 46.9% of the resected patients with HCC. Interestingly, the frequency of OGH was higher in the patients with than those without HCC among the liver biopsy patients, although no significant difference was noted. It has been reported that HCC develops commonly in cirrhotic patients, but rarely in non-cirrhotic patients.[16,17] HCC has been reported to develop in as high as 3–10% of hepatitis C-related cirrhotic patients yearly.[18,19] A more advanced stage of fibrosis has been reported to be associated with a higher incidence of complication by HCC. Considering that OGH frequently developed in HCC patients in association with a long duration of infection, the stage of fibrosis and the appearance of OGH in biopsy tissue may serve as indicators predicting the development of HCC.

Hypotheses about the pathogenesis of OGH have been proposed.[20–25] As immunohistochemical staining showed no differences among zones in the expression of Ki-67, which reflects the cell growth cycle, it is difficult to consider that OGH is regenerated hepatocytes. Tanji *et al.*[26] performed histochemical studies of OGH in chronic hepatitis, and found deficiencies of mtDNA and mtDNA-encoded respiratory chain enzymes in OGH. We evaluated the function of the increased mitochondria, and obtained results different from theirs. Histochemical staining and immunohistochemical staining with antimitochondrial antibody showed increased activity of electron transport enzymes in the

area of increased mitochondria, indicating that there was no functional impairment of the hyperplastic mitochondria. In addition, the increased staining for HXK-II suggests an increased glycolytic pathway. The increase in the glycolytic pathway and the increased activity of electron transport enzymes indicate that energy production was markedly enhanced. In this study, we did not perform DNA analysis, a factor to be studied in the future. The presence of areas of increased and markedly decreased mitochondria suggests that the former area represents compensatory mitochondrial hyperplasia for the latter area. OGH may be an extreme form of compensatory hyperplastic change.

REFERENCES

1 Lefkowitch JH, Arborgh BA, Scheuer PJ. Oxyphilic granular hepatocytes. Mitochondrion-rich liver cells in hepatic disease. *Am J Clin Pathol* 1980; 74: 432–41.
2 Gerber MA, Thung SN. Hepatic oncocytes. Incidence, staining characteristics, and ultrastructural features. *Am J Clin Pathol* 1981; 75: 498–503.
3 Altmann HW. [Oncocytic hepatocytes.] *Pathologe* 1990; 11: 137–42 (in German).
4 Muller-Hocker J. Defects of the respiratory chain in hepatic oncocytes. *Virchows Arch* 1998; 432: 349–56.
5 Kage M, Shimamatu K, Nakashima E *et al.* Long-term evolution of fibrosis from chronic hepatitis to cirrhosis in patients with hepatitis C: morphometric analysis of repeated biopsies. *Hepatology* 1997; 25: 1028–31.
6 Ludwig J. The nomenclature of chronic active hepatitis: an obituary. *Gastroenterology* 1993; 105: 274–8.
7 Stoward PJ, Pearse AGE. Histochemical methods for dehydrogenases. In: Stoward PJ, Pearse AGE, eds. *Histochemistry. Vol. 3 Enzyme Histochemistry*, 4th edn. London: Churchill Livingstone, 1991; 537–72.
8 Woltsche M, Zatloukal K, Denk H. Enzyme-histochemical studies of griseofulvin-intoxicated mouse livers. *Liver* 1991; 11: 231–40.
9 Chieco P, Moslen MT, Reynolds ES. Histochemical evidence that plasma and mitochondrial membranes are primary foci of hepatocellular injury caused by 1,1-dichloroethylene. *Lab Invest* 1982; 46: 413–21.
10 Kang YH, McKenna T, Watson LP *et al.* Cytochemical changes in hepatocytes of rats with endotoxemia or sepsis: localization of fibronectin, calcium, and enzymes. *J Histochem Cytochem* 1988; 36: 665–78.
11 Yasuda S, Arii S, Mori A *et al.* Hexokinase II and VEGF expression in liver tumors: correlation with hypoxia-inducible factor 1 alpha and its significance HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. *J Hepatol* 2004; 40: 117–23.
12 Deeb SS, Malkki M, Laakso M. Human hexokinase II: sequence and homology to other hexokinases. *Biochem Biophys Res Commun* 1993; 197: 68–74.
13 Shibata M, Morizane T, Uchida T *et al.* Irregular regeneration of hepatocytes and risk of hepatocellular carcinoma in chronic hepatitis and cirrhosis with hepatitis-C virus infection. *Lancet* 1998; 351: 1773–7.
14 Uchida T. Pathology of hepatitis C. *Intervirology* 1994; 37: 126–32.
15 Uchida T, Kronborg I, Peters RL. Acute viral hepatitis: morphologic and functional correlations in human livers. *Hum Pathol* 1984; 15: 267–77.
16 Colombo M. Natural history and pathogenesis of hepatitis C virus related hepatocellular carcinoma. *J Hepatol* 1999; 31 (Suppl 1): 25–30.
17 De Mitri MS, Poussin K, Baccarini P *et al.* HCV-associated liver cancer without cirrhosis. *Lancet* 1995; 345: 413–15.
18 Marcellin P. Hepatitis C: the clinical spectrum of the disease. *J Hepatol* 1999; 31 (Suppl 1): 9–16.
19 Alberti A, Chemello L, Benvegnu L. Natural history of hepatitis C. *J Hepatol* 1999; 31 (Suppl 1): 17–24.
20 Bassi M, Bernelli-Zazzera A. Ultrastructural cytoplasmic changes of liver cells after reversible and irreversible ischemia. *Exp Mol Pathol* 1964; 17: 332–50.
21 Confer DB, Stenger RJ. The evolution of lysosomes in hypoxic liver parenchyma as seen with the electron microscope. *Am J Pathol* 1964; 45: 533–46.
22 Oudea PR. Anoxic changes of liver cells. Electron microscopic study after injection of colloidal mercury. *Lab Invest* 1963; 12: 386–94.
23 Jordan SW. Electron microscopy of hepatic regeneration *Exp Mol Pathol* 1964; 86: 183–200.
24 Herdson PB, Garvin PJ, Jennings RB. Fine structural changes produced in rat liver by partial starvation. *Am J Pathol* 1964; 45: 157–81.
25 Lafontaine JG, Allard C. A light and electron microscope study of the morphological changes induced in rat liver cells by the azo dye 2-Me-Dab. *J Cell Biol* 1964; 22: 143–72.
26 Tanji K, Bhagat G, Vu TH *et al.* Mitochondrial DNA dysfunction in oncocytic hepatocytes. *Liver Int* 2003; 23: 397–403.

Toll-like Receptor Expression in Lupus Peripheral Blood Mononuclear Cells

KIYOSHI MIGITA, TAICHIRO MIYASHITA, YUMI MAEDA, MINORU NAKAMURA, HIROSHI YATSUHASHI, HIRONORI KIMURA, HIROMI ISHIBASHI, and KATSUMI EGUCHI

ABSTRACT. ***Objective.*** To investigate expression of members of the Toll-like receptor (TLR) family in peripheral blood mononuclear cells (PBMC) in patients with systemic lupus erythematosus (SLE).
Methods. We analyzed PBMC from 14 patients with SLE and 15 healthy subjects. The surface expressions of TLR2 and TLR4 and intracellular expression of TLR9 on PBMC were analyzed by flow cytometry.
Results. Although TLR4 expressions on CD14+ monocytes were not significantly different between healthy subjects and patients with SLE, TLR2 expressions on monocytes were reduced in patients with SLE compared to healthy subjects. Intracellular TLR9 expression levels of CD19+ B lymphocytes were significantly elevated in patients with SLE. However, the TLR9 expression levels of plasmacytoid dendritic cells were not significantly different between these patients and healthy subjects.
Conclusion. Our results show that human peripheral blood B cells express TLR9 and that its expression is increased in patients with SLE. This upregulated expression of TLR9 in B cells may be related to the abnormal B cell hyperactivity in patients with SLE. (First Release Jan 15 2007; J Rheumatol 2007;34:493–500)

Key Indexing Terms:
B LYMPHOCYTES
INTERFERON-α
SYSTEMIC LUPUS ERYTHEMATOSUS
TOLL-LIKE RECEPTOR

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the generation of autoantibodies against nuclear proteins and DNA[1]. These autoantibodies are thought to contribute to the pathogenesis of SLE, and the levels of anti-DNA antibodies correlate with the disease activity[2,3]. The deposition of antinuclear antibody and antigen immune complexes (IC) in tissue is thought to induce local activation of immune systems[4], which may perpetuate SLE. It has also been proposed that the type 1 interferon (IFN) system has a pivotal etiopathogenic role in SLE, since IFN-α correlates with disease activity of SLE[5,6]. Recent studies showed that the sera of patients with SLE selectively induce the production of IFN-α from the natural IFN-α-producing cells, which are identical to plasmacytoid dendritic cells (PDC)[7]. Further, DNA/anti-DNA antibody complexes in lupus sera can provide signals for IFN-α production through the Toll-like receptor-9 (TLR9), which responds to bacterial DNA sequences containing nonmethylated CpG motifs[8]. In humans, the TLR family consists of 10 members, each of which is involved in the recognition of pathogen-derived materials[9]. Recent data suggest that TLR also play an important role in autoimmunity[10]. It is important to determine whether circulatory mononuclear cells in patients with SLE express TLR that could be involved in PDC activation as well as IFN-α induction. We examined the expression of TLR in lupus circulating mononuclear cells using flow cytometry methods.

From the Clinical Research Center and Department of General Internal Medicine, NHO Nagasaki Medical Center, Omura; and First Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan.

K. Migita, MD, Clinical Research Center and Department of General Internal Medicine; Y. Maeda, MSc; M. Nakamura, MD; H. Yatsuhashi, MD; H. Ishibashi, MD, Clinical Research Center; T. Miyashita, MD; H. Kimura, MD, Department of General Internal Medicine, NHO Nagasaki Medical Center; K. Eguchi, MD, First Department of Internal Medicine, Nagasaki University School of Medicine.

Address reprint requests to Dr. K. Migita, Clinical Research Center, NHO Nagasaki Medical Center, Kubara 2-1001-1, Omura 856-8652, Japan. E-mail: migita@nmc.hosp.go.jp

Accepted for publication October 25, 2006.

MATERIALS AND METHODS

Patients and controls. A total of 14 patients with SLE (12 women and 2 men, aged 36.1 ± 13.3 yrs) were enrolled in our study (Table 1), in addition to 15 healthy volunteers (12 women, 3 men, aged 37.7 ± 8.1 yrs) and 6 patients with rheumatoid arthritis (RA; 5 women, 1 man, aged 48.1 ± 11.4 yrs) as controls. Consecutive patients entering the rheumatology clinic who fulfilled the American College of Rheumatology 1982 revised classification criteria for SLE[11] were selected for this investigation. Among the 14 patients, 8 received prednisolone (PSL) as monotherapy (mean dosage 20.3 mg/day, range 5–60 mg/day). The remaining 5 patients were treated with both PSL (mean dosage 19.4 mg/day, range 7-50 mg/day) and cyclophosphamide (intermittent intravenous cyclophosphamide therapy), and one patient received PSL (5 mg/day) and azathioprine (50 mg/day). Disease activity was scored in all patients with SLE by SLE Disease Activity Index (SLEDAI)[12]. The study protocol was approved by the Ethics Committee of Nagasaki Medical Center.

Reagents for flow cytometry. Antibodies used for phenotype analysis were obtained from Beckman Coulter (Fullerton, CA, USA), and antibodies for TLR analysis were purchased from eBioscience (San Diego, CA, USA). Antibody for BDCA-2 was purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Antibodies used in this study were as follows: FITC-labeled anti-human CD14, CD19, and BDCA-2; phycoerythrin (PE)-labeled anti-human TLR2, 4, and 9; and PC-5-labeled anti-human CD123.

Flow cytometry. Heparinized blood was collected and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation. For staining for TLR9, PBMC were stained for cell surface antigen and fixed

Table 1. Clinical data of patients with SLE.

Patient No.	Age, yrs	Sex	SLEDAI	Anti-dsDNA, IU/ml	CH50, IU/ml	PSL, mg/day	Cyclophosphamide	Azathioprine
1	49	F	6	141	30	25		
2	27	F	4	26	30	11		
3	29	F	9	17	12	14		
4	19	F	5	5	36	5		+
5	27	F	8	21	29	10		
6	24	F	14	30	15	30		
7	57	F	4	5	38	7	+	
8	45	F	12	33	25	12.5	+	
9	25	F	16	14	26	60		
10	33	F	12	19	23	50	+	
11	38	F	2	17	38	5		
12	55	M	10	32	41	15	+	
13	24	M	18	17	29	15	+	
14	54	F	10	4	37	7.5		

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; anti-dsDNA: anti-double-stranded DNA antibody (normal 0.0–10.0 IU/ml); PSL: prednisolone.

and permeabilized using a commercial kit (eBioscience). Cells were then stained for intracellular TLR9 with a PE-labeled TLR9 monoclonal antibody (mAb; eBioscience). In brief, cells were stained for their surface markers using fluorescein isothiocyanate (FITC)-conjugated anti-human CD14, CD19, blood dendritic cell antigen (BDCA)-2, or PC-5-conjugated CD123 for 15 min. Cells were then washed with staining buffer, fixed, and permeabilized. After washing with staining buffer, cells were stained with PE-conjugated anti-human TLR9 (eBioscience). Fluorescence was measured on an Epics XL (Beckman-Coulter). The acquired data were analyzed with EXPO32 software (Beckman-Coulter).

Cell culture. PBMC were isolated by Ficoll-Hypaque (Amersham Pharmacia, Tokyo, Japan) density gradient centrifugation. T cells were removed from PBMC by negative selection using anti-CD2 magnetic beads (Dynal, Oslo, Norway). T-cell-depleted mononuclear cells (MNC; 1×10^5 per 200 μl well) were cultured in RPMI-1640 (Gibco, NY, NY, USA) supplemented with 10% fetal bovine serum (Gibco BRL Invitrogen, Tokyo) in 96-well tissue culture plates. CpG oligodeoxynucleotide (ODN) 2006 (5'-TCG TCG TTT TGT CGT TTT GTC GTT-3') complete phosphorothioate (CpG ODN2006; InvivoGen, San Diego, CA, USA) was added at Day 0. T-cell-depleted MNC were also cultured with 96-well plates coated with 1 μg/ml purified goat anti-human IgG, IgA, and IgG (Southern Biotechnology, Birmingham, AL, USA) to trigger the B cell receptor (BCR) as controls. Cell cultures were maintained for 4 days and culture supernatants were collected and stored at –20°C. The amounts of IgG in culture supernatants were determined by antibody sandwich-type ELISA (Bethyl Laboratories, Montgomery, AL, USA).

Statistical analysis. Comparisons between groups were done using the nonparametric Mann-Whitney U-test. All statistics were performed with Stat View 7.0 (SAS Institute, Cary, NC, USA).

RESULTS

TLR2 and TLR4 expressions on CD14+ cells in patients with SLE. We compared the baseline expression of TLR2 and TLR4 on CD14+ monocytes in patients with SLE and healthy subjects. As shown in a representative histogram of monocytes, surface expressions of TLR2 were lower in patients with SLE than in healthy subjects (Figure 1A). The geometric mean fluorescence intensity of TLR2 was significantly lower in patients with SLE than in healthy subjects (Figure 1B). However, there was no statistical difference in TLR4 expression between patients with SLE and healthy subjects (Figures 2A, 2B). Although we also examined the baseline expression of TLR2 and TLR4 on B cells and T cells in patients with

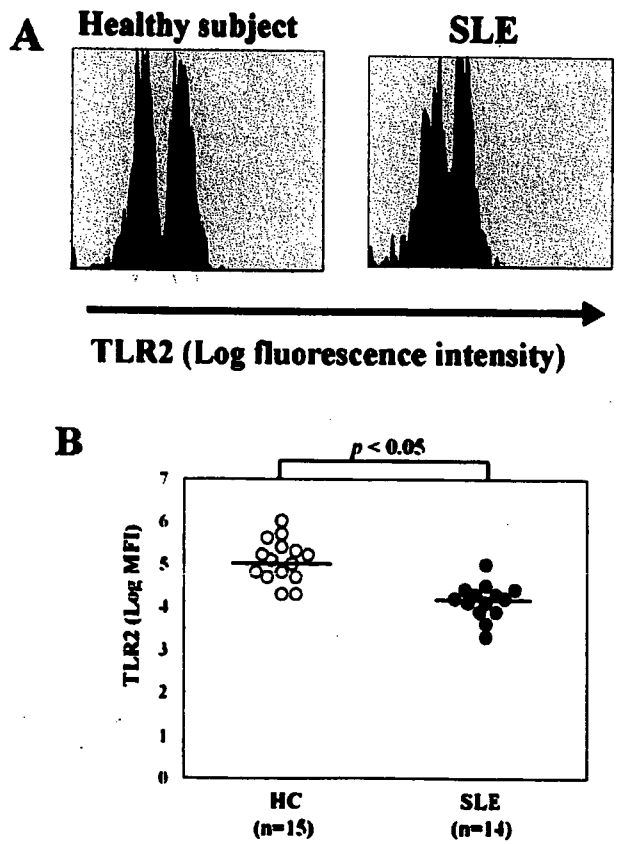


Figure 1. Diminished expression of TLR2 on CD14+ monocytes in patients with SLE. A. Histograms show baseline expression of surface TLR2 on monocytes in a representative patient with SLE and healthy subject. Darker areas indicate staining with isotype control mAb. B. Mean fluorescence intensity of surface TLR2 staining for CD14+ monocytes in patients with SLE (n = 14) and healthy subjects (HC; n = 15). Significance of differences between groups were analyzed by Mann-Whitney U-test.

SLE, we could not detect TLR2 and TLR4 expression on B or T cells in patients with SLE or in healthy subjects (data not shown).

Increased expression of TLR9 on B cells in patients with SLE. We also examined whether lupus B cells express TLR9 in patients with SLE. We identified B cells as CD19+ populations (Figures 3A, 3B). As shown in Figure 3C, CD19+ B cells express TLR9 constitutively. In a representative histogram of B cells, intracellular expression of TLR9 was higher in patients with SLE than in healthy subjects. The geometric mean fluorescence intensity of TLR9 in B cells is shown in Figure 3D. TLR9 expression levels in B cells were significantly increased in patients with SLE compared to healthy subjects. In contrast, these increased expressions of TLR9 in B cells were not observed in the patients with RA, another autoimmune disease (Figures 4A, 4B). We evaluated the relationship between TLR9 expression on B cells and the treatment regimens in patients with SLE. However, there was no significant difference of TLR9 expression in the presence or absence of moderate doses of corticosteroids (PSL > 20 mg/day) or immunosuppressants (Figure 5). Similarly, there was no significant difference of TLR2 expression on CD14+ monocytes in the presence or absence of moderate doses of corticosteroids or immunosuppressants (Figure 6). We also evaluated the correlations of the TLR9 expression levels in B cells with clinical measures such as the SLEDAI, hemolytic complement (CH50), and titers of anti-dsDNA antibodies. However, there was no significant correlation between TLR9 expression levels and these clinical measures.

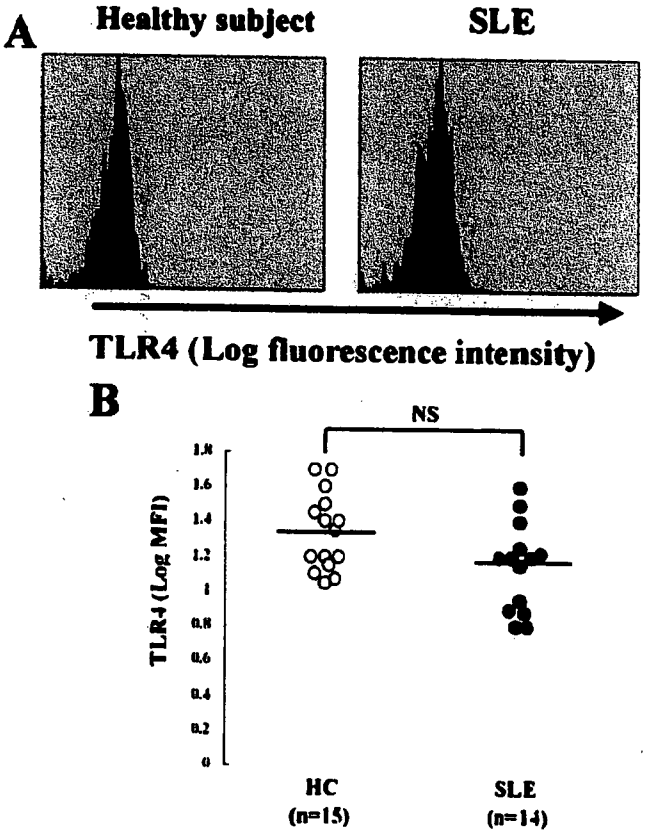


Figure 2. Expression of TLR4 on CD14+ monocytes in patients with SLE. A. Histograms show baseline expression of surface TLR4 on monocytes in a representative patient with SLE and healthy subject. Darker areas indicate staining with isotype control mAb. B. Mean fluorescence intensity of surface TLR2 staining for CD14+ monocytes in patients with SLE (n = 14) and healthy subjects (HC: n = 15). Significance of differences between groups were analyzed by Mann-Whitney U-test.

TLR9 expression in lupus PDC. It was demonstrated that TLR9 mediates the activation of PDC by SLE-IC. To investigate TLR9 expression on PDC, we performed double-staining of PBMC, with a mixture of mAb against CD123 and BDCA-2. PDC, identified as CD123/BDCA-2 double-positive populations[13], were gated (Figure 7) and analyzed for TLR9 expression. In order to make low proportions of peripheral blood PDC visible especially in patients with SLE, at least 100,000 cells were acquired. Figure 6A shows a representative result for the TLR9 expression of gated BDCA-2+, CD123+ cells from healthy subjects and patients with SLE. No significant difference between healthy subjects and patients with SLE was observed with regard to the TLR9 expression on PDC (Figure 8).

IgG production following CpG stimulation in B cells. To determine the ability of CpG ODN to stimulate immunoglobulin production by lupus B cells, T-cell-depleted MNC were cultured in the presence of CpG ODN. Cultured supernatants were harvested on Day 5 of culture and analyzed for the presence of IgG by ELISA. CpG ODN stimulated the production of IgG from control and lupus B cells. The increase in IgG production was 1.6-fold by control B cells, and an equivalent 1.9-fold increase in lupus B cells. This difference was not statistically significant. Under stimulation through BCR, the increase in IgG production was 5.6-fold in controls and 6.4-fold in SLE, and no significant difference was observed. These findings indicate that lupus B cells respond to CpG ODN stimulation; however, the ability to respond to CpG ODN in lupus B cells was not different from that in control B cells (Table 2, Figure 9).

DISCUSSION

Type 1 IFN has been proposed to have a pivotal etiopathogenic role in SLE, since serum levels of IFN-α correlate with the disease activity of SLE[5,6]. IFN-α may contribute to lupus autoimmune processes by differentiating B lymphocytes and following autoantibody production[14]. TLR are a family of pattern-recognition receptors that evolved to detect microbial infection[9]. These receptors recognize conserved molecular products derived from different classes of microorganisms[15]. Although TLR detect molecular patterns of microbial origin, some TLR and their ligands have emerged as important regulators of immunity relevant to effector responses to autoimmunity[16].

Recent data indicate that immune complexes containing

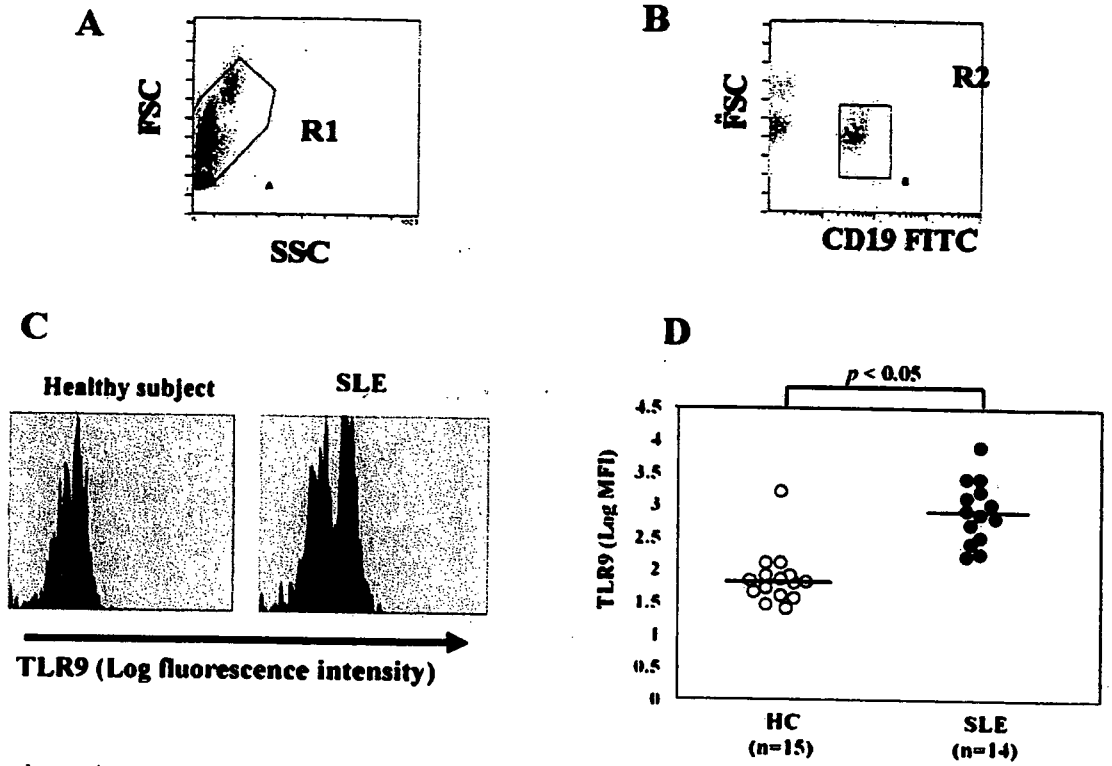
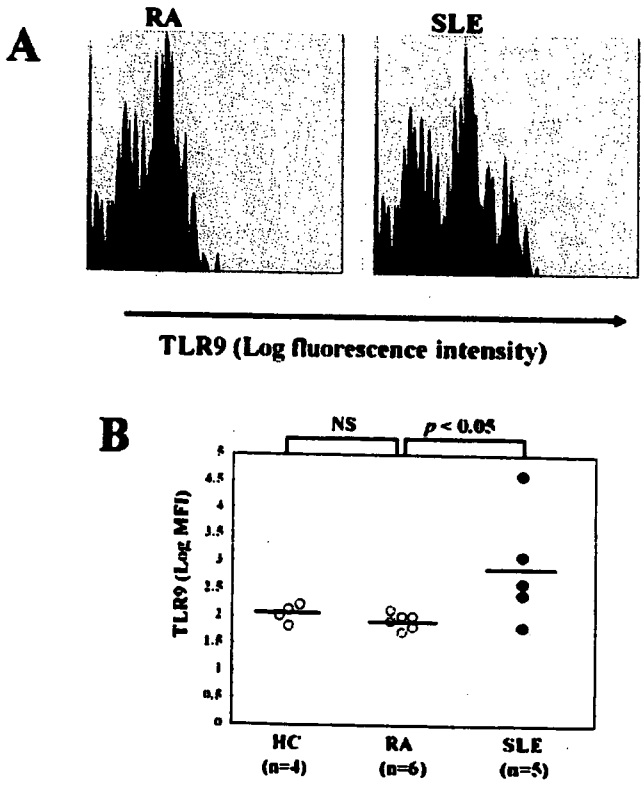


Figure 3. Upregulated expression of TLR9 on CD19+ B cells in patients with SLE. A, B. Isolation of CD19+ B cells by flow cytometry. PBMC were assessed by forward scatter (FS) and side scatter (SS) measures. Cells gated in R1 (A) were further analyzed for anti-CD19 FITC-labeled mAb. CD19+ cells were gated (R2) and defined as B cells (panel B). C. Histograms depicting baseline expression of intracellular TLR9 on B cells in a representative patient with SLE and healthy subject. Darker areas indicate staining with isotype control mAb. D. Mean fluorescence intensity of intracellular TLR9 staining for CD19+ B cells in patients with SLE (n = 14) and healthy subjects (HC; n = 15). Significance of differences between groups were analyzed by Mann-Whitney U-test.



nucleic acid and lupus IgG can induce IFN-α secretion from PDC[8]. Further, Means, *et al* showed that SLE-IC stimulates PDC to produce IFN-α in a TLR9-dependent manner[17]. The ability of PDC to secrete IFN-α depends on cellular sensors that detect the presence of bacterial DNA or IC[18], and TLR9 expression may account for the IFN-α production from PDC. We investigated the expression of TLR9 on circulating PDC in patients with SLE. Our data show that the expression of TLR9 in PDC was not different between patients with SLE and healthy subjects. Previous investigations showed that the numbers of circulating PDC are decreased in patients with SLE[19], but large numbers of activated PDC infiltrate skin lesions and actively produce IFN-α[20]. These findings suggest that PDC seem to be activated by IC in the pathogenic lesions and circulating PDC may not reflect these immune conditions. Further investigations concerning the TLR9 expression in tissue-infiltrating PDC are needed in patients with SLE.

Figure 4. Expression of TLR9 on CD19+ B cells in patients with RA and SLE. A. Histograms show baseline expression of intracellular TLR9 on B cells in representative patients. Darker areas indicate staining with isotype control mAb. B. Mean fluorescence intensity of intracellular TLR9 staining for CD19+ B cells in healthy subjects (HC, n = 4), patients with RA (n = 6), and with SLE (n = 5). Significance of differences between groups were analyzed by Mann-Whitney U-test.

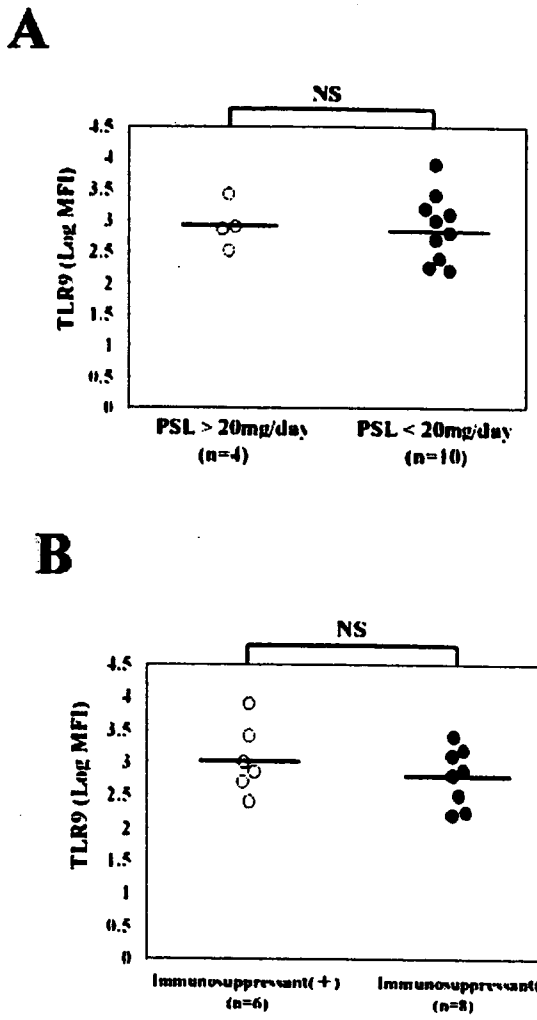


Figure 5. Expression of TLR9 on CD19+ B cells in patients with SLE. A. Mean fluorescence intensity (MFI) of intracellular TLR9 staining for CD19+ B cells in patients with SLE treated with > 20 mg/day prednisolone (PSL; n = 4) or < 20 mg/day PSL (n = 10). Significance of differences between groups were analyzed by Mann-Whitney U-test. B. MFI of intracellular TLR9 staining for CD19+ B cells in patients with SLE treated with (n = 6) or without immunosuppressants (n = 8). Significance of differences between groups were analyzed by Mann-Whitney U-test.

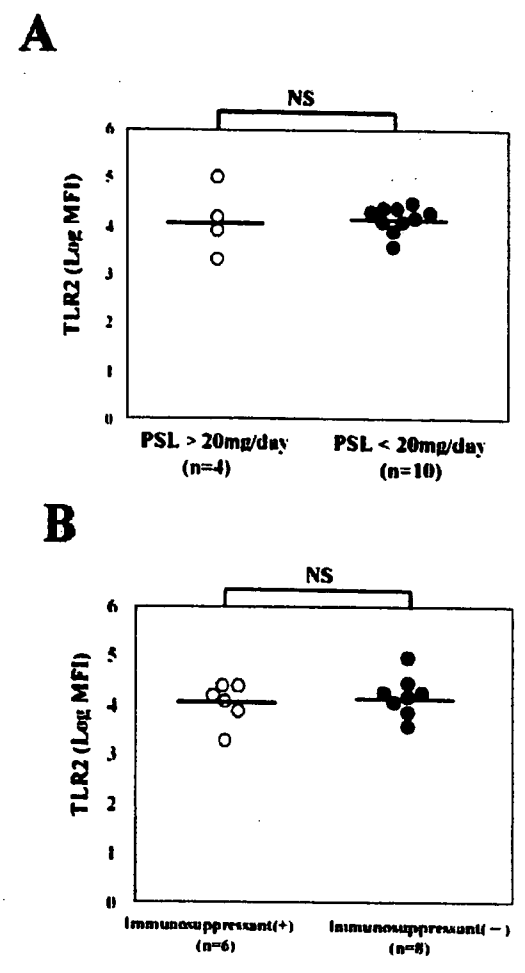


Figure 6. Expression of TLR2 on CD14+ monocytes in patients with SLE. A. Mean fluorescence intensity (MFI) of intracellular TLR2 staining for CD14+ B monocytes in patients with SLE treated with > 20 mg/day prednisolone (PSL; n = 4) or < 20 mg/day PSL (n = 10). Significance levels for differences between groups were analyzed by Mann-Whitney U-test. B. MFI of intracellular TLR2 staining for CD14+ monocytes in patients with SLE treated with (n = 6) or without immunosuppressants (n = 8). Significance of differences between groups were analyzed by Mann-Whitney U-test.

The high serum levels of IFN-α in patients with SLE were found to promote the differentiation of B cells as well as to activate PDC[21]. Similar to PDC, B cells express a limited set of TLR, including TLR7 and TLR9[22]. Leadbetter, *et al* showed that autoreactive B cells are activated by IgG-chromatin IC and require the synergistic engagement of B cell receptor and TLR9[23]. It is postulated that engagement of the BCR by an autoantibody-antoantigen IC triggers the endocytosis of IC that then results in the efficient delivery of chromatin fragments to endosome-associated TLR9[23]. We consistently observed the expression of TLR9 in freshly isolated human peripheral blood B cells, and our first major observation was the upregulated expression of TLR9 in peripheral blood B cells from patients with SLE. More recently, it was postulated that the engagement of TLR receptors, particularly TLR9, on B cells seems to play an important role in B cell activation and autoantibody production[24]. The importance of the TLR9-dependent pathways will depend on the levels of TLR9 expression. The differential expression of TLR9 may correlate with the responsiveness to CpG DNA, and the altered TLR9 expression could potentially affect the B cell immune response to chromatic or IC in patients with SLE.

CpG ODN have been shown to activate B cells via the family of TLR9[25]. Our data indicated that both control and lupus B cells responded to CpG DNA stimulation. The ability of lupus B cells to produce IgG was higher compared to control B cells; however, the difference was not significant. Since our B cell preparations contain mononuclear cells (T-cell-depleted mononuclear cells), the abnormal response of non-B cell pop-

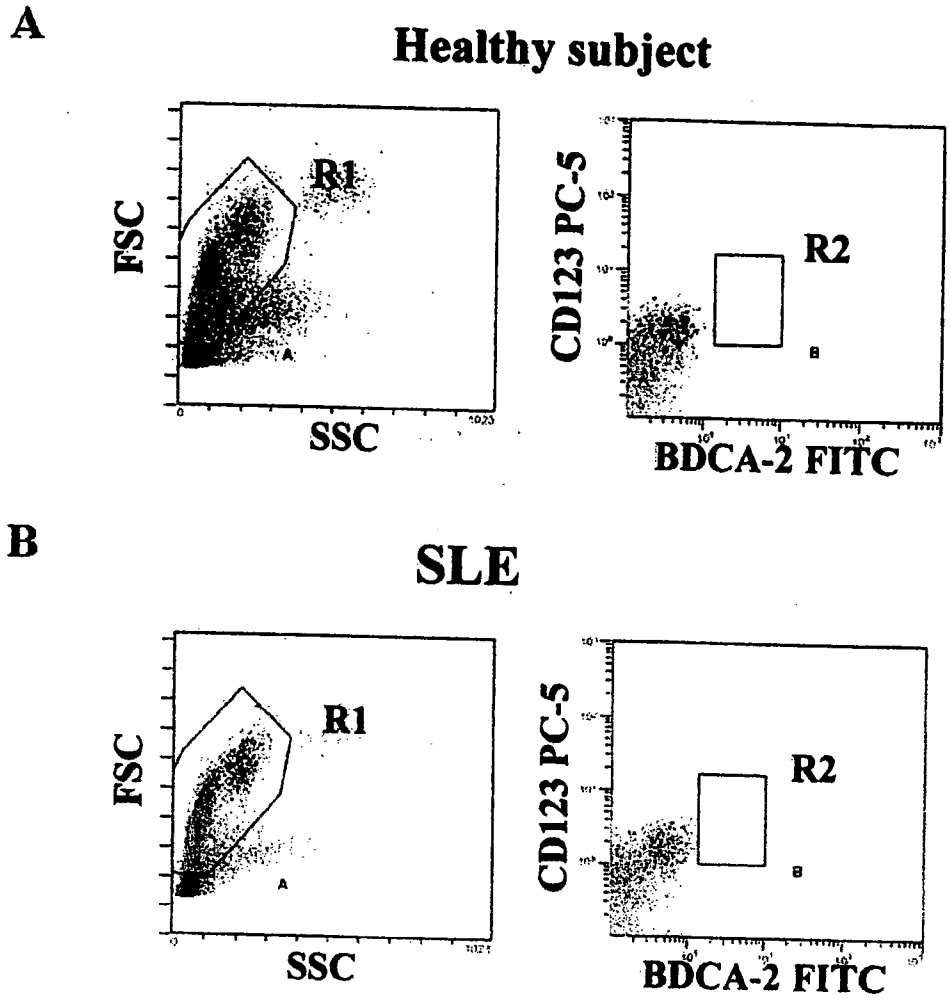


Figure 7. Identification of circulating plasmacytoid dendritic cells (PDC) by flow cytometry in peripheral blood from a representative patient with SLE and healthy subject. Mononuclear cells isolated from a healthy subject (A) and patient with SLE (B) were assessed by forward scatter (FS) and side scatter (SS). The R1-gated events were then analyzed for BDCA-2 and CD123, and BDCA-2+, CD-123+ double-positive cells (R2) were defined as PDC.

ulations to CpG DNA contributed to this phenomenon as described previously[26].

The exact immune mechanisms underlying the upregulation of TLR9 on lupus B cells remain to be elucidated in our study. Bernasconi, *et al* indicated that in human naive B cells, TLR9 is expressed at low to undetectable levels, but its expression is rapidly upregulated by BCR triggering[27]. In contrast, memory B cells expressed TLR9 at constrictively high levels[28]. SLE is characterized by polyclonal B cell activation[29], and these alternations of B cell activation or differentiation status may account for the upregulation of TLR9 expression. We could not show the interaction between increased TLR9 expression on B cells and lupus disease activity. Our study included 14 patients with SLE with low to moderate disease activity; therefore, further large-scale investigations of patients with SLE are needed to elucidate the relationship between TLR9 expression on B cells and lupus disease activity.

In summary, we showed that TLR9 expression in B lymphocytes was increased in patients with SLE. This upregulated TLR9 expression may activate B lymphocytes through the interaction between TLR9 and its ligands and may be related to the pathogenesis of lupus.

REFERENCES

1. Ruiz-Irastorza G, Khamashta MA, Castellino G, Hughes GR. Systemic lupus erythematosus. Lancet 2001;357:1027-32.
2. Hahn BH. Antibodies to DNA. N Engl J Med 1998;338:1359-68.
3. ter Borg EJ, Horst G, Hummel EJ, Limburg PC, Kallenberg CG. Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. A long-term, prospective study. Arthritis Rheum 1990;33:634-43.
4. Ebling FM, Hahn BH. Pathogenic subsets of antibodies to DNA. Int Rev Immunol 1989;5:79-95.
5. Ronnblom L, Alm GV. An etiopathogenic role for the type I IFN system in SLE. Trends Immunol 2001;22:427-31.

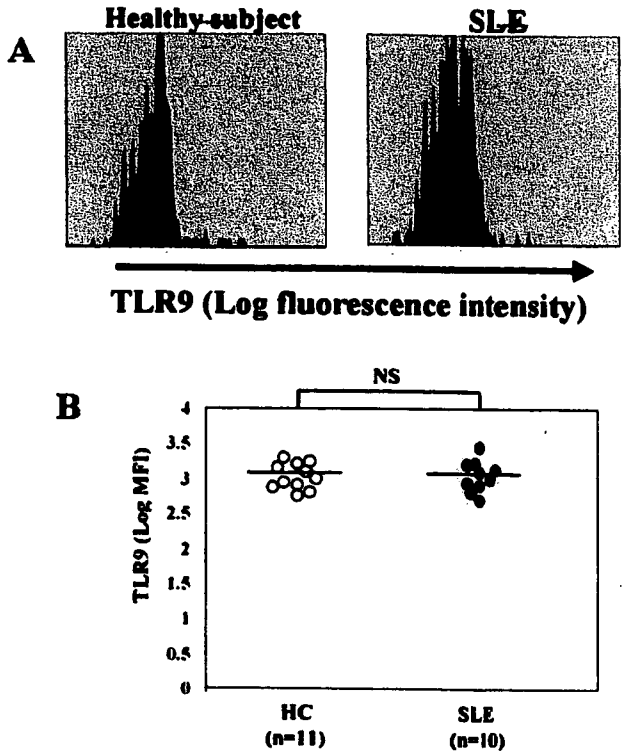


Figure 8. Expression of TLR9 on plasmacytoid dendritic cells (PDC) in patients with SLE. A. Histograms show baseline expression of intracellular TLR9 on PDC in a representative patient with SLE and healthy subject. Darker areas indicate staining with isotype control mAb. B. MFI of surface TLR9 staining for PDC in patients with SLE (n = 14) and healthy subjects (HC; n = 15). Significance of differences between groups were analyzed by Mann-Whitney U-test.

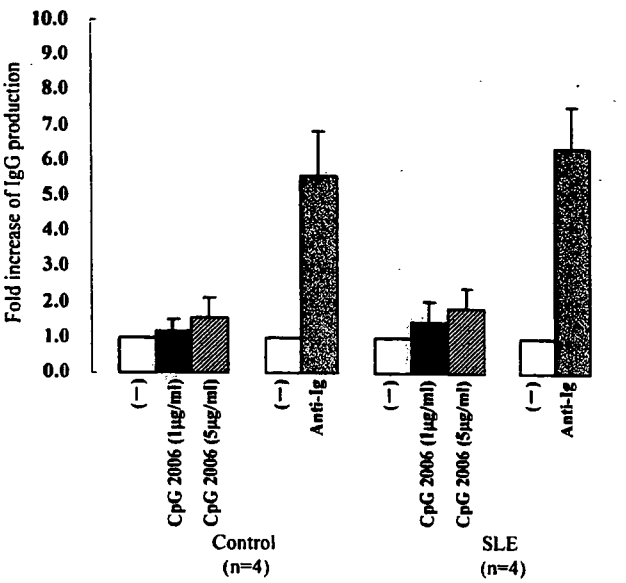


Figure 9. Secretion of IgG by PBMC from patients with SLE (n = 4) and healthy subjects (control; n = 4) after stimulation with CpG ODN. Freshly isolated T cell-depleted MNC were cultured with CpG ODN and anti-IgG. The amounts of IgG in culture supernatants were determined by ELISA. The IgG secreted from untreated MNC was assigned the value of 1.0 and data were expressed as fold induction. These data represent mean ± SD of 4 independent samples.

Table 2. Immunoglobulin production after 4 days of culture with CpG oligodeoxynucleotide. Results are mean (SD) of duplicate samples.

	IgG, ng/ml			
	None	CpG 2006 (1 μg/ml)	CpG 2006 (5 μg/ml)	Anti-Ig
Control				
1	125.2 (21.7)	135.1 (17.4)	212.6 (17.9)*	417.2 (44.7)*
2	106.2 (9.5)	155.5 (25.1)*	195.4 (32.1)*	517.2 (69.1)*
3	97.6 (11.6)	105.6 (9.9)	121.5 (17.8)*	617.5 (55.2)*
4	79.1 (14.8)	85.5 (13.2)	96.5 (9.5)	715.2 (69.9)*
SLE				
1	105.4 (7.2)	252.4 (19.2)*	289.2 (17.7)*	781.2 (91.1)*
2	132.4 (16.3)	167.5 (12.1)	245.1 (21.9)*	824.6 (102.9)*
3	108.8 (8.9)	118.2 (9.4)	162.5 (12.7)*	654.9 (78.1)*
4	152.7 (11.1)	191.9 (17.1)*	233.5 (29.1)*	932.7 (115.4)*

* $p < 0.05$ vs No stimulation.

6. Ronnblom L, Alm GV. Systemic lupus erythematosus and the type I interferon system. Arthritis Res Ther 2003;5:68-75.
7. Ronnblom L, Alm GV. A pivotal role for the natural interferon alpha-producing cells (plasmacytoid dendritic cells) in the pathogenesis of lupus. J Exp Med 2001;194:59-63.
8. Lovgren T, Eloranta ML, Bave U, Alm GV, Ronnblom L. Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. Arthritis Rheum 2004;50:1861-72.
9. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335-76.
10. Pasare C, Medzhitov R. Toll-like receptors: linking innate and adaptive immunity. Adv Exp Med Biol 2005;560:11-8.
11. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
12. Urowitz MB, Gladman DD. Measures of disease activity and

damage in SLE. Baillieres Clin Rheumatol 1998;12:405-13.
13. Dzionek A, Fuchs A, Schmidt P, et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J Immunol 2000;165:6037-46.
14. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. Type 1 interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity 2001;14:461-70.
15. Kopp E, Medzhitov R. Recognition of microbial infection by Toll-like receptors. Curr Opin Immunol 2003;15:396-401.
16. Rifkin IR, Leadbetter EA, Busconi L, Viglianti G, Marshak-Rothstein A. Toll-like receptors, endogenous ligands, and systemic autoimmune disease. Immunol Rev 2005;204:27-42.
17. Means TK, Latz E, Hayashi F, Murali MR, Golenbock DT, Luster AD. Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. J Clin Invest 2005;115:407-17.
18. Bauer S, Wagner H. Bacterial CpG-DNA licenses TLR9. Curr Top Microbiol Immunol 2002;270:145-54.
19. Scheinecker C, Zwolfer B, Koller M, Manner G, Smolen JS. Alterations of dendritic cells in systemic lupus erythematosus: phenotypic and functional deficiencies. Arthritis Rheum 2001;44:856-65.
20. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon-alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol 2001;159:237-43.
21. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol 2005;23:275-306.
22. Hornung V, Rothenfusser S, Britsch S, et al. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 2002;168:4531-7.
23. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 2002;416:603-7.
24. Viglianti GA, Lau CM, Hanley TM, Miko BA, Shlomchik MJ, Marshak-Rothstein A. Activation of autoreactive B cells by CpG dsDNA. Immunity 2003;19:837-47.
25. Bauer S, Kirschning CJ, Hacker H, et al. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. Proc Natl Acad Sci USA 2001;98:9237-42.
26. Zeuner RA, Klinman DM, Illei G, et al. Response of peripheral blood mononuclear cells from lupus patients to stimulation by CpG oligodeoxynucleotides. Rheumatology Oxford 2003;42:563-9.
27. Bernasconi NL, Onai N, Lanzavecchia A. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. Blood 2003;101:4500-4.
28. Kikuchi K, Lian ZX, Yang GX, et al. Bacterial CpG induces hyper-IgM production in CD27(+) memory B cells in primary biliary cirrhosis. Gastroenterology 2005;128:304-12.
29. Renaudineau Y, Pers JO, Bendaoud B, Jamin C, Youinou P. Dysfunctional B cells in systemic lupus erythematosus. Autoimmun Rev 2004;3:516-23.

ウイルス肝炎の臨床へのアプローチ
その2

肝癌撲滅をめざしたウイルス肝炎の治療戦略

八橋　弘
国立病院機構長崎医療センター臨床研究センター治療研究部／やつはし・ひろし

はじめに

わが国には，約150万人から200万人のHCV（C型肝炎ウイルス）キャリア，約100万人のHBV（B型肝炎ウイルス）キャリアが存在すると推定されている．一方わが国の年間肝癌死亡者数は3万4千人を超し，主要悪性新生物死亡順位で，男性では第3位，女性では第4位に位置している．その肝癌死亡者の約80%がHCV感染，約10%がHBV感染に由来する．わが国ではHCV感染者は非感染者に比較して千倍以上，HBV感染者は百倍以上の肝癌リスクを有する．わが国のウイルス感染対策は肝癌対策であるといい換えても過言ではなく，ウイルス肝炎治療の目標，治療介入目的は，これらの肝炎ウイルス感染者の肝癌抑止，肝癌撲滅にある．しかしながら，これらの肝炎ウイルスキャリアが一様に発癌するのではなく，個々の患者により発癌リスクは大きく異なる．

本稿では，HCV感染，HBV感染の自然経過を振り返りながら，個々の患者の発癌リスクを正確に理解したうえで，ウイルス排除と発癌抑止の観点から，治療介入の意義を考察する．

C型肝炎の自然経過

図1に約20年の経過でC型慢性肝炎から肝硬変へ進展し発癌した症例を示す．この症例では観察期間の20年間に10回肝生検を施行したが，その所見の推移をstage（肝線維化stage）とactivity（炎症の程度）に分けて示している．まずstageの推移は初回肝生検時F1，以後9年間はF1のままで推移するも1985年から1988年にかけてF1からF2，F4（肝硬変）に急速に進行し，肝硬変進展5年目に発癌した．一方，activityの推移は，ALTの推移と連動しながらstageの変化に先行する形で持続的に上昇し肝硬変進展後は逆に低下した．C型慢性肝炎進展例の典型的経過とは，図1の症例のように30歳代，40歳代の慢性肝炎の初期の時期にはALT値の変動にもかかわらず肝線維化stageの進行はみられないが，50歳を超えた時期から急速に肝線維化stageが進行して肝硬変へ移行し，60歳前後で発癌する．

C型慢性肝炎の自然経過，発癌リスクを，肝線維化stageを指標に表現したものが図2である．すなわち，HCV持続感染者は，肝機能障害すなわち肝細胞の破壊と再生を繰り返しながら，F0からF1，F2，F3，F4（肝硬変）へと20年から30年以上かけてゆっくり階段を昇るように進展し，最終的には肝硬変を経由して肝癌が発生する．自験例での肝線維化stage別年間発癌率の成績は，F0：1.2%，F1：1.3%，F2：3.4%，F3：5.7%，F4：7.8%であり，stageの進展とともに肝発癌のリスクも上昇する．肝線維化stageは，発癌の確率，発癌までの時間を推定する上で有用な指標であり，HCV持続感染者は可能な限り肝生検を行って発癌のリスクを把握することが望ましい．肝生検が困難な場合は，血小板数，肝線維化マー

1345-1551/07/¥100/頁/JCLS

- *わが国には，約150万人から200万人のHCVキャリア，約100万人のHBVキャリアが存在する*
- *肝癌死亡者の約80%がHCV感染，約10%がHBV感染に由来する．*
- *非感染者に比較してHCV感染者は千倍以上，HBV感染者は百倍以上の肝癌リスクを有する．*

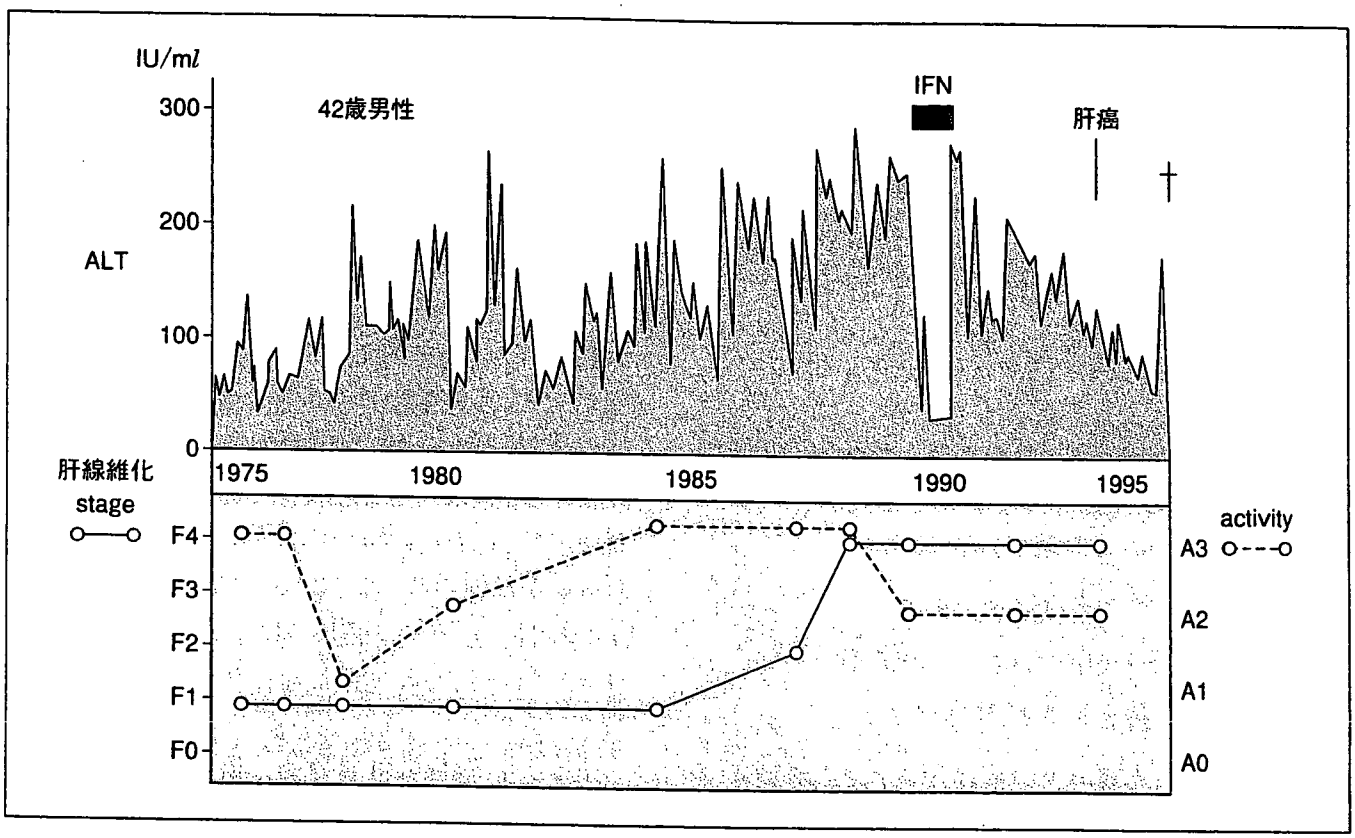


図1　C型慢性肝炎から肝硬変，肝癌へと進展した1例

カーを測定することで，肝線維化stageの推定はある程度は可能である．

一方，C型慢性肝炎から肝硬変進展に寄与する因子として以下の項目が報告されている[1]．① 感染時年齢が40歳以上，② 男性，③ 過剰なアルコール飲酒(エタノール換算で1日50g以上)，④ 輸血による感染(薬物常用者と比較して)，⑤ HBV，HIVとの重複感染などである．C型慢性肝炎患者が自然治癒することはきわめてまれではあるが，C型慢性肝炎患者が全員，肝硬変，肝癌に進展するのではないことも明らかとなっている．Poynardら[1]によると，C型慢性肝炎患者の33%は20年以内に肝硬変に進展するも，31%は肝硬変に進展しないか進展するとしても50年

- *肝線維化 stage は，発癌の確率，発癌までの時間を推定する上で有用な指標となる．*
- *C 型慢性肝炎が自然治癒することはきわめてまれである．*
- *C 型慢性肝炎患者の約 30% は肝硬変に進展し，一度肝硬変に進展した場合は年率 7～8% の確率で肝癌が発生する．*

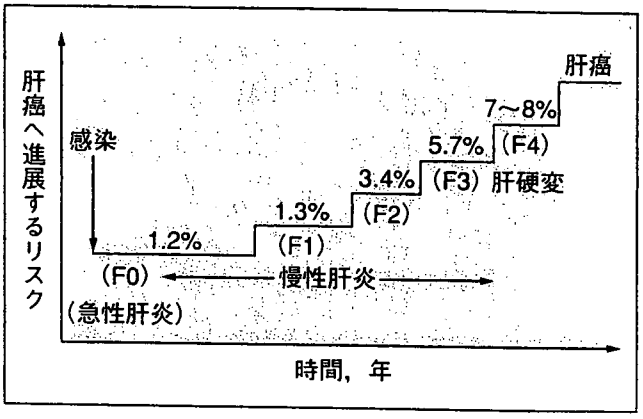


図2　C型慢性肝炎の自然経過，線維化stageと年間発癌率

以上の時間を要すると推定している．

C 型肝炎の治療目標，治療戦略とインフォームドコンセント

C 型慢性肝炎患者の自覚症状は乏しく慢性肝炎のまま進展しないことが約束されるならば，C 型慢性肝炎患者の生命予後，QOL は健常者と同等と考えられる．しかし，C 型慢性肝炎が自然治癒することはきわめてまれで，慢性肝炎患者の約 30% は 20 年から 30 年の経過のうちに肝硬変に進展し，一度肝硬変に進展した場合は年率 7～8% の確率で肝癌が発生する．その進展の仕方は一様でなく，特に年齢の因子によって進展速度が異なる．一般に C 型慢性肝炎の進展速度は，50 歳まではほとんど進展がみられないかきわめて緩徐だが，50 歳前後ごろから急速に肝線維化が進展，肝硬変へと移行する．肝癌発生の平均年齢は 60～65 歳である．また，多量飲酒により肝線維化の進展速度は早まり肝硬変へ移行する確率が高まるとともに肝癌発生までの期間が短縮することも指摘されている．

C 型慢性肝炎という病気を患者に理解させ説明するポイントとして，① 慢性肝炎のまま進展せず発癌しなければ生命予後は健常者と同じである，② C 型慢性肝炎が自然治癒することはきわめてまれである，③ C 型慢性肝炎患者の約 30% は 20～30 年の経過で肝硬変に進展する，④ 肝硬変進展後は高率に癌化する，⑤ 多量飲酒は病気の進展を加速する，などがあげられる．上記の内容を説明した上で，通院，検査の必要性を説き，禁酒，節酒および規則正しい生活など指導を行う．

さらに C 型慢性肝炎患者に対しては可能な限り肝生検を施行して肝線維化 stage を明らかにする．肝線維化 stage を把握することで，慢性肝炎の進展度，発癌のリスクが明確となり，個々の患者に対して細やかな治療方針を決定することがはじめて可能となる．

インターフェロン(IFN)療法を行う場合には，その治療目的を明確に患者に説明しなければならない．すなわち ① ウイルス駆除を目的とする，② 発癌抑止を目的とする，といった説明である．IFN は高価な薬剤で副作用の出現率が高いなどのデメリットを有する一方で，C 型慢性肝炎の治

● *C型肝炎に対するIFN治療目標は，ウイルス駆除，発癌抑止の二つに大別される．*

● *IFN少量長期投与法は，ウイルスの排除を目的とせず，肝線維化進展抑止，発癌抑止を目的とした治療である．ALT値，AFP値を治療効果指標とする．*

癒，進展阻止を可能にするというメリットも有している．IFN療法に関する患者への説明としては，IFN療法をすることのメリット，デメリット，IFN療法をしないことのメリット，デメリットを患者に説明した上で，IFN療法に関して患者からインフォームドコンセントを得なければならない．そのためには，個々の患者の肝線維化stageから現在の慢性肝炎の進展度，発癌のリスクを明確にし，またウイルス量，HCV遺伝子型やHCVサブグループからIFN治療効果を予測する．また各IFN治療法の特性を十分に理解することが必要である．さらに個々の患者ごとに予期される副作用も十分考慮した上で，これらの因子を総合的に評価し，最終的にIFN療法の是非と具体的な治療法を選択，決定する．

進展抑止，発癌抑止をめざしたC型肝炎に対するIFN治療(IFN少量長期療法と肝硬変に対するIFN治療)

ペグIFN/Rib併用療法無効例，副作用の観点からペグIFN/Rib併用療法の導入が困難な高齢者，発癌リスクの高い肝線維化高度進展例に対する新たな治療法としてIFN少量長期投与法が試みられている．ウイルスの排除を目的とせず，肝線維化進展抑止，発癌抑止を目的とした治療である．その治療の理論背景は以下の二つに大別される．① IFNを抗炎症剤として用いて，ALT値の持続正常化，肝線維化進展抑止を目指す．② IFNには，細胞増殖抑制効果があり，肝癌細胞にも作用してその増殖を抑制する，アポトーシスを誘導する，新生血管発育を抑制する，といった作用がある．①を治療目標とする場合にはALT値を指標とし，②の場合にはAFPなどの腫瘍マーカーを指標として，それぞれの治療法の効果を評価する．

また，2004年4月からC型肝硬変患者に対するIFN βの投与が，一部の患者において保険診療上可能となった．C型肝硬変患者のウイルス駆除を目的とするも開発試験の成績では，仮にIFN β投与でウイルスが排除されなくとも持続的にALT値が低いbiochemical responder例では肝癌発生が長期に抑止されたことが示されている．

B型肝炎の自然経過

HBVそのもの自体は細胞障害性を有していない．HBV持続感染は，ウイルス，肝細胞，宿主免疫機構の三つの因子のバランスの中で成立する．HBV持続感染者の自然経過は，ウイルスの量，炎症所見，年齢などを考慮した上で，1) **immune tolerance期**，2) **immune clearance期**，3) **low replicative期またはintegrated期**という三つの時相に分類される(**図3**)[2]．

1. immune tolerance期

この時期は，いわゆる肝機能正常のHBe抗原陽性無症候性キャリアとしてとらえられている．HBV増殖は活発でHBV-DNA量も最も高い時期

● *B型肝炎の自然経過は，1) immune tolerance 期，2) immune clearance 期，3) low replicative 期 の三つの時相に分類される．*

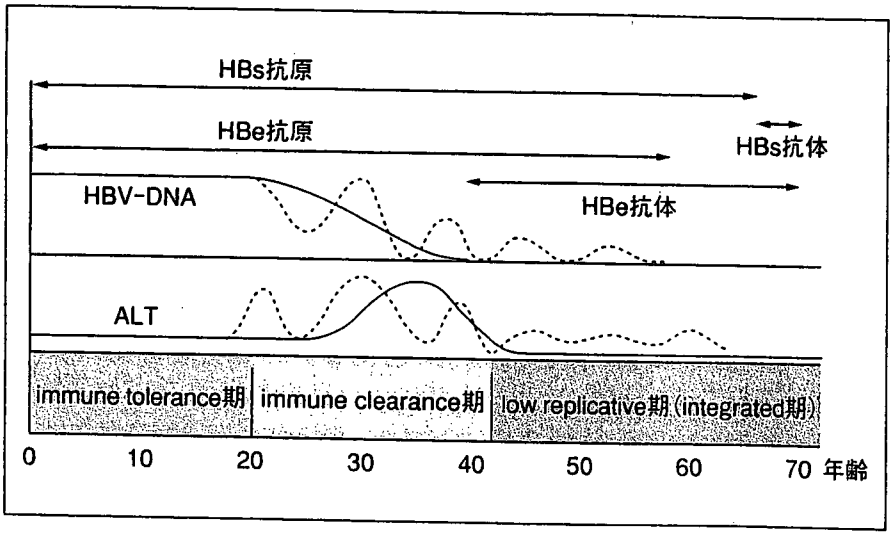


図3　HBV持続感染者の自然経過
(文献2) より)

だが，ウイルス抗原に対する宿主サイドの認識が成立していない免疫寛容の状態であることから，肝組織所見は正常か，炎症所見はあってもきわめて軽微である．垂直感染でHBV持続感染が成立した場合，15歳前後までこのような時期が持続する．

2.　**immune clearance 期**

HBVに対する感染個体の免疫応答，細胞性免疫が活性化しサイトカインなどの誘導により肝炎が発症する時期である．著しい肝機能障害を呈しながらHBV感染肝細胞が徐々に排除され，HBV増殖が低下しHBV-DNA量が減少する時期である．いわゆる慢性肝炎の時期であり，年齢としては15歳から35歳の時期に相当する．この免疫排除の時期に，激しい肝炎が生じた場合には患者の中には肝不全症状を呈することがある．激しい肝炎の後には，しばしばHBe抗原-HBe抗体のセロコンバージョンが認められる．

肝組織所見では慢性肝炎時期の炎症が軽い場合は軽度の線維化を残すのみで治癒するが，激しい炎症が10年前後ほど長期間持続した場合，肝線維化は高度となり肝硬変に進展する．肝炎の急性増悪に伴う肝小葉改築の程度と広がりとその持続期間が，肝疾患の予後を決定すると考えられる．

3.　**low replicative 期(integrated 期)**

HBVに対する宿主免疫が優位になり，肝臓のほとんどのウイルス感染細胞が排除され，HBe抗原陰性，HBe抗体陽性のセロコンバージョンが持続しALT値も持続正常を示す時期である．この時期ではHBV増殖は停止もしくは著しく低

● *無症候性キャリアからの年間の肝硬変進展率は約 1%，B 型慢性肝炎からの年間の肝硬変進展率は約 2% で累積肝硬変進展率は 5.5 年で 15% と報告されている．*

● *年間肝癌進展率は HBV 無症候性キャリアでは 0.1～0.4%，B 型慢性肝炎では 0.5～0.8%，肝硬変では 3～10% と報告されている．*

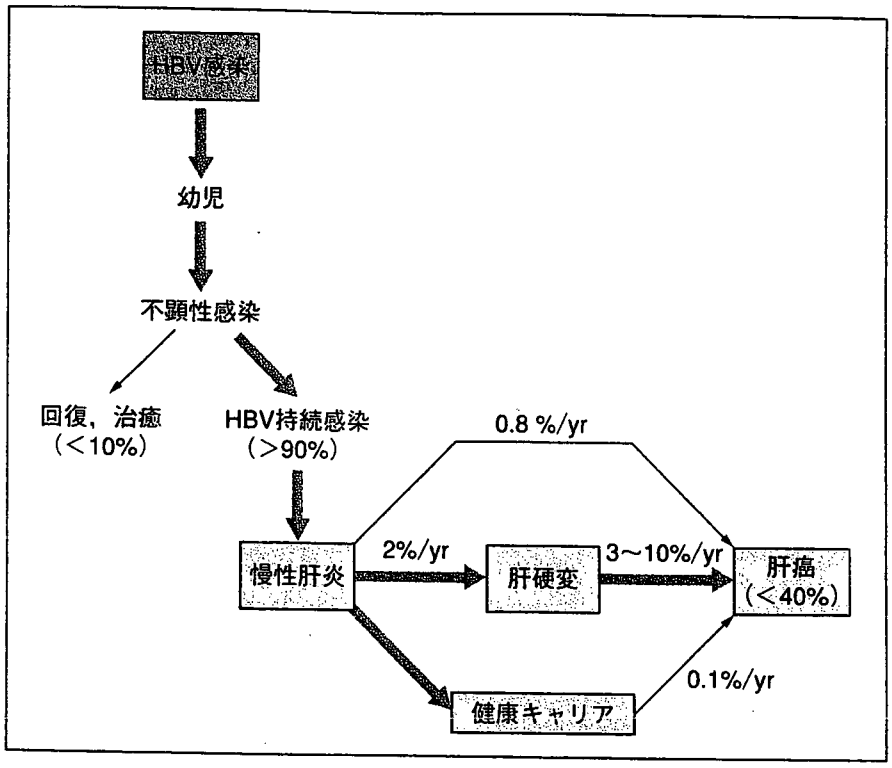


図4　幼少期にHBV持続感染が成立した者の長期自然経過
(文献4) より)

下し HBV-DNA は PCR 法によってのみ検出される程度である．肝細胞のゲノム内の HBs 遺伝子が組み込み，もしくは微量のウイルスの存在により HBs 抗原は陽性となる．肝組織所見では炎症所見は鎮静化しており，HBe 抗原の再出現や，HBe 抗原陰性 HBV-DNA 陽性の HBV 変異ウイルスによる活動性肝炎の場合などの例外を除くと，一般的には B 型肝炎の臨床的寛解とみなされる．

この時期を経過した後，一部の患者では，HBs 抗原が消失し，HBs 抗体陽性の integrated 期となる．HBV キャリア成人例における HBs 抗原の年間消失率は約 1% である．

B 型肝炎からの肝硬変と肝癌への進展

immune clearance 期の長さと炎症の重症度によって肝硬変進展が規定される．無症候性キャリアからの年間の肝硬変進展率は約 1%，B 型慢性肝炎からの年間の肝硬変進展率は約 2% で累積肝硬変進展率は 5.5 年で 15% と報告されている．

● *肝癌進展の危険因子として，1) 年齢，2) ウイルスの増殖状態(HBV-DNA量，HBe抗原の有無)，3) 肝硬変の有無，4) 性差，5) 飲酒，6) 喫煙，などが報告されている．*

● *B型肝炎の治療効果の判定の項目は，ALT値の正常化，HBeAgの消失，HBeAgのセロコンバージョン，HBV-DNAの陰性化(non-PCR)，肝組織所見の改善などである．*

表1　B型慢性肝炎に対する抗ウイルス療法の新しい治療効果判定基準

category of response	
biochemical (BR)	血清ALT値が正常範囲内に低下すること
virologic (VR)	血清HBV-DNA量が，非増幅法で検出されないレベル(<10^5copies/ml)にまで低下し，治療前HBeAg陽性例ではHBeAgが陰性化すること
histologic (HR)	治療前の肝生検所見と比較して，組織活動性スコアーが少なくとも2ポイント以上低下していること
complete (CR)	BRとVRの基準を満たし，かつHBsAgが消失していること
time of assessment	
on-therapy	治療期間中
maintained	治療中の期間
end of treatment	治療終了時点
off-therapy	治療終了後
sustained (SR-6)	治療終了後6ヵ月の期間
sustained (SR-12)	治療終了後12ヵ月の期間

(文献3)より)

肝硬変進展の危険因子としては，1)亜急性の肝不全症状や組織所見でbridging necrosisを伴う肝炎の急性増悪，2)患者の年齢(40歳以上)，が重要であり40歳以上でbridging necrosisを伴う肝炎の場合は肝硬変に進展しやすいも，若年の患者ではbridging necrosisを伴う肝炎であってもその後HBe抗原-HBe抗体のセロコンバージョンが得られれば組織変化が改善することが多いといわれている[3]．

HBV持続感染者は肝癌進展のハイリスク群であり，Taiwanでは非感染者に比較して98～223倍のリスクがあると報告されている．年間肝癌進展率は無症候性キャリアでは0.1～0.4%，B型慢性肝炎では0.5～0.8%，肝硬変では3～10%と報告されている(図4)[4]．肝癌進展の危険因子として，① 年齢，② ウイルスの増殖状態(HBV-DNA量，HBe抗原の有無)，③ 肝硬変の有無，④ 性差，⑤ 飲酒，⑥ 喫煙，などが報告されている[3,4]．

B型肝炎の治療目標，効果判定，治療戦略

HBV感染では，HCV感染とは異なり，その複雑な感染，ウイルス増殖様式から体内からのHBVの完全排除は困難である．B型慢性肝炎の治療目標はHBV増殖の持続抑制と肝病変の改善とする考えかたが基本となる．治療効果の判定の

● *ウイルス肝炎での肝発癌の条件，プロセスをまとめると 1) HBV，HCV 持続感染である，2) 炎症を伴う，3) 肝硬変をベースとして高率に発癌する，の3点に集約される．*

● *慢性肝炎から肝癌への進展を阻止する方法として，1) HBV，HCV 持続感染を断ち切る，2) 炎症を鎮静化させる，3) 肝硬変に進展させない，ことなどが発癌阻止に有効である．*

項目として，ALT 値の正常化，HBeAg の消失，HBeAg のセロコンバージョン，HBV-DNA の陰性化(non-PCR)，肝組織所見の改善などがあげられる(表1)．B 型肝炎の治療法としては，IFN とラミブジン，アデホビル，エンテカビルなどの核酸誘導体からなる抗ウイルス剤に大別される．

ウイルス肝炎から肝癌への進展阻止

肝癌発生機序として従来からいくつかの仮説が唱えられているが，疫学的，臨床的な観点からウイルス肝炎での肝発癌の条件，プロセスをまとめると ① HBV，HCV 持続感染である，② 炎症を伴う，③ 肝硬変をベースとして高率に発癌する，の3点に集約される．よって慢性肝炎から肝癌への進展を阻止する方法としては，① まず原因となる HBV，HCV 持続感染を断ち切る．② ウイルス排除が困難な場合には炎症を鎮静化させることを目指す．いずれにしても ③ 各種薬剤治療により前癌病変ともいえる肝硬変に進展させないことが発癌阻止に有効である．

文　献

1) Poynard, T., Bedossa, P., Opolon, P.：Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet **349**：825-832, 1997
2) Lok, A. S. F.：Treatment of chronic hepatitis B. J Viral Hep **1**：105-124, 1994
3) Lok, A. S. F., McMahon, B. J.：Chronic hepatitis B. Hepatology **34**：1225-1241, 2001
4) Chu, C. M.：Natural history of chronic hepatitis B virus infection in adults with emphasis on the occurrence of cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol **15** (suppl)：25-30, 2000