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Peripheral CD5$^+$B Cells Reflect Regional Immunity of the Liver in Pediatric Cholestatic Diseases

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Summary. Certain immunological abnormalities are thought to be the causative factors in pediatric cholestatic diseases (PCD). However, few studies have been done regarding the simultaneous analysis of the lymphocyte phenotype of the liver and the peripheral blood (PBL) in PCD. To understand better the immunological background of PCD, we analyzed the lymphocyte subpopulations in the liver and in the PBL. Ten patients with PCD were investigated in this study: four patients with biliary atresia (BA), five patients with congenital biliary dilatation, and one patient with a paucity of interlobular bile ducts. Both liver tissue and peripheral blood samples were obtained during a total of 11 incidences of surgery on these patients. The collected samples were analyzed with a FACScan. The expressions of CD4, CD8, CD20, CD56, CD5$^-$CD20$^+$ (CD5$^-$B cells) and CD5$^+$CD20$^+$ (CD5$^+$B cells) surface antigens were evaluated in the liver and PBL. The relative size of the lymphocyte subpopulations of CD4, CD8 and CD56 in PBL respectively differed from those in the liver significantly. No significant correlation was observed between the relative amount in the PBL and that of the liver regarding each marker. The relative size of the CD5$^+$B cells in the PBL was nearly same as that in the liver, and only CD5$^+$B cells showed a significant positive correlation between these cells in the PBL and liver. The CD5$^+$B cell subpopulation in the PBL may provide valuable, non-invasive information regarding the regional immunity of the liver in patients with PCD.

Key words— cholestatic liver disease, CD5$^+$B cell, children, immunological abnormality, lymphocyte subpopulation.

INTRODUCTION

In adult liver diseases such as HCV hepatitis, the presence of lymphocytes in the liver has been considered to be associated with the pathogenesis.[1)] In pediatric cholestatic diseases (PCD), including biliary atresia (BA), some immunological abnormalities are thought to be the causative factors.[2,3,4)] Non-invasive makers — which reflect the regional immunity of the liver, are thus desirable as it is not easy to obtain liver specimens during the follow-up period in pediatric patients. To understand better the immunological background of PCD, we performed a flow cytometric analysis of the lymphocyte subpopulations in the liver and peripheral blood (PBL) simultaneously. The aime of this study was to evaluate the possibility that some PBL lymphocyte markers may help to provide valuable, non-invasive information regarding the regional immunity of the liver in patients with PCD.

PATIENTS AND METHODS

We examined samples from ten patients with PCD in this study: four patients with BA, five patients with congenital biliary dilatation, and one patient with a paucity of interlobular bile ducts. The patient profiles are summarized in Table 1. Both liver biopsy specimens and

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Abbreviations– BA, biliary atresia; PBL, peripheral blood; PCD, pediatric cholestatic disease; TB, serum total bilirubin level.

heparinized PBL samples were simultaneously obtained during each operation.

All subjects were investigated after obtaining full parental informed consent to participate in this study. This study carefully complied with the Helsinki Declaration of 1975 (revised 2000).

PBL and liver mononuclear cells were prepared as previously described.[5] A phenotypic analysis was performed using Fluorescein isothiocyanate-, phycoerythrin-, or Cychrome-conjugated monoclonal antibodies. The cells were incubated with the relevant monoclonal antibodies for 30 min at 4 °C, and then were washed and analyzed on a FACScan (Becton-Dickinson, Mountain View, CA, USA). The expressions of CD4, CD8, CD56, $CD5^{-}CD20^{+}$, and $CD5^{+}CD20^{+}$ surface antigens were evaluated in the liver and PBL. Both the CD4 and CD8 mean T cell markers and CD56 signify the natural killer cells (NK cell) marker. The surface marker of $CD5^{-}CD20^{+}$ signifies conventional B cells and is dominant in human adult PBL ($CD5^{-}$B cell). The surface marker of $CD5^{+}$ $CD20^{+}$ indicates specific B cells, which also express the T cell antigen. This B cell is called a Murine Ly-1 B cell ($CD5^{+}$B cells).[6]

We compared the relative size of each lymphocyte subpopulation for both the PBL and the liver. We also investigated the correlation between the relative size of each lymphocyte subpopulation in the PBL with that in the liver. A comparison of the lymphocyte subpopulations in the PBL and in the liver was analyzed using the Mann-Whiteney U test. Spearman's rank correlation test was performed for a two-factor correlation. Differences were considered to be significant if the ρ value was less than 0.05.

RESULTS

The relative size of the lymphocyte subpopulations in the liver and PBL are summarized in Table 2. The relative sizes of the lymphocyte subpopulations of $CD4^{+}$, CD8, $CD56^{+}$ in PBL were significantly different from those in the liver. On the other hand, there were no significant differences regarding both the $CD5^{-}$B cells and $CD5^{+}$ B cells. Only the relative size of the lymphocyte subpopulations of $CD5^{+}$ B cells showed a significantly positive correlation between those in the PBL and in the liver ($r = 0.714$, $p = 0.011$) (Fig. 1).

In one patient with BA (patient 1), the expression of $CD5^{+}$ B cells was dramatically lower than that of the other patients in both the PBL and the liver at a re-do Kasai's operation for his age (total bilirubin (TB) 10.3 mg/dl and ALT 328 IU/L; 0.5% and 1.9%, respectively). On the other hand, in another patient with BA (patient 2), the expression of $CD5^{+}$ B cells in the PBL and liver remained unchanged regardless of the presence of jaundice, i.e. before Kasai's operation with jaundice (TB 6.4 mg/dl and ALT 178 IU/L; 14.2% and 21.9%, respectively) and after Kasai's operation without jaundice (TB < 1 mg/dl and ALT 49 IU/L; 15.8% and 16.7%, respectively) (Fig. 2).

DISCUSSION

Possible defects of the immune system in patients with PCD can be evaluated by estimating the functionally different lymphocyte subpopulation in the PBL. Socha et al.[3] demonstrated that a deficiency in the expression of the $CD45RA^{+}$ $CD4^{+}$ T cell ('naive' $CD4^{+}$ cells) was an important finding in PCD. However, this study was performed by analyzing only the PBL, and the lymphocyte subpopulation in the liver of PCD patients has not yet been analyzed. To our knowledge, our study is the first to make a simultaneous flow cytometric analysis of the lymphocyte subpopulations in the liver and in the PBL in PCD.

Because of its location and function, the liver is continuously exposed to a large number of antigens and required to cope with these diverse immunological challenges.[7] Therefore, local resident hepatic lymphocytes are thought to play a crucial role in the mechanisms of regional immunity. In a previous study in

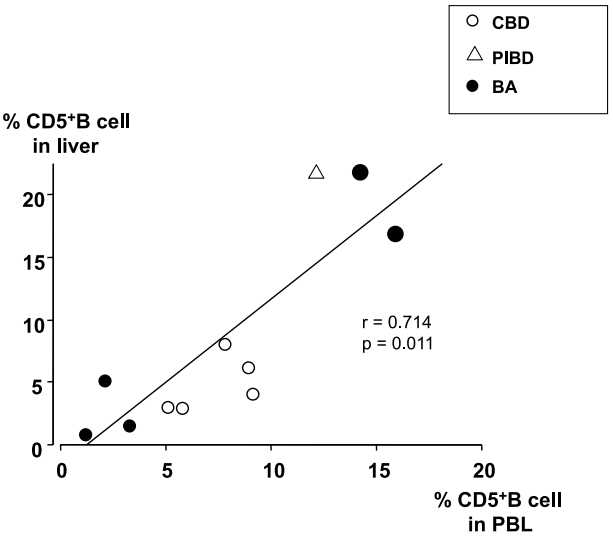

Fig. 1. Correlation of the relative size of $CD5^{+}$ B cells in PBL with that in the liver. A positive correlation was found between the percentage of $CD5^{+}$ B cells in the PBL and that in the liver. BA, biliary atresia; CBD, congenital biliary dilatation; PBL, peripheral blood; PIBD, paucity of interlobular bile duct.

Table 1. Summary of the patient profiles

Patient	Age (months)	Primary disease	TB at this study (mg/dl)	Surgery when speciemens were obtained
1	3	BA	10.3	Re-do Kasai's operation
2-1	2	BA	6.4	Kasai's operation
2-2	7	BA	< 1.0	Anti-reflux surgery after Kasai's operation
3	210	BA	< 1.0	Liver biopy
4	204	BA	25.7	LRLT
5	27	CBD	< 1.0	Radical surgery
6	60	CBD	< 1.0	Radical surgery
7	69	CBD	< 1.0	Radical surgery
8	15	CBD	< 1.0	Radical surgery
9	144	CBD	< 1.0	Radical surgery
10	2	PIBD	5.4	Exploratory laparotomy

BA, biliary atresia; CBD, congential biliary dilatation; LRLT, living related liver transplantation; PIBD, paucity of interlobular bile duct; TB, total bilirubin; Radical surgery, choledochocystectomy with cholesystectomy and Roux-en Y reconstruction.

Table 2. Relative sizes of lymphocyte subpopulations in the PBL and liver

			p value	
Marker of the phenotype	PBL (n = 11)	Liver (n = 11)	Mean	Correlation between PBL and the liver
CD4	48.3 ± 10.8% (31 - 71%)	18.2 ± 8.3% (11- 41%)	0.004	NS
CD8	23.5 ± 7.4% (15 - 42%)	33.2 ± 13.2% (13 - 50%)	0.012	NS
CD56	10.0 ± 8.2% (2 - 30%)	29.9 ± 11.2% (11 - 52%)	0.008	NS
$CD5^-CD20^+$	10.8 ± 4.8% (5 - 18%)	8.5 ± 6.6% (2 - 20%)	NS	NS
$CD5^+CD20^+$	7.6 ± 5.0% (0.5 -16%)	8.5 ± 7.8% (1.6 - 22%)	NS	0.011

Data are expressed as the mean ± SD (range); NS, not significant; PBL, peripheral blood.

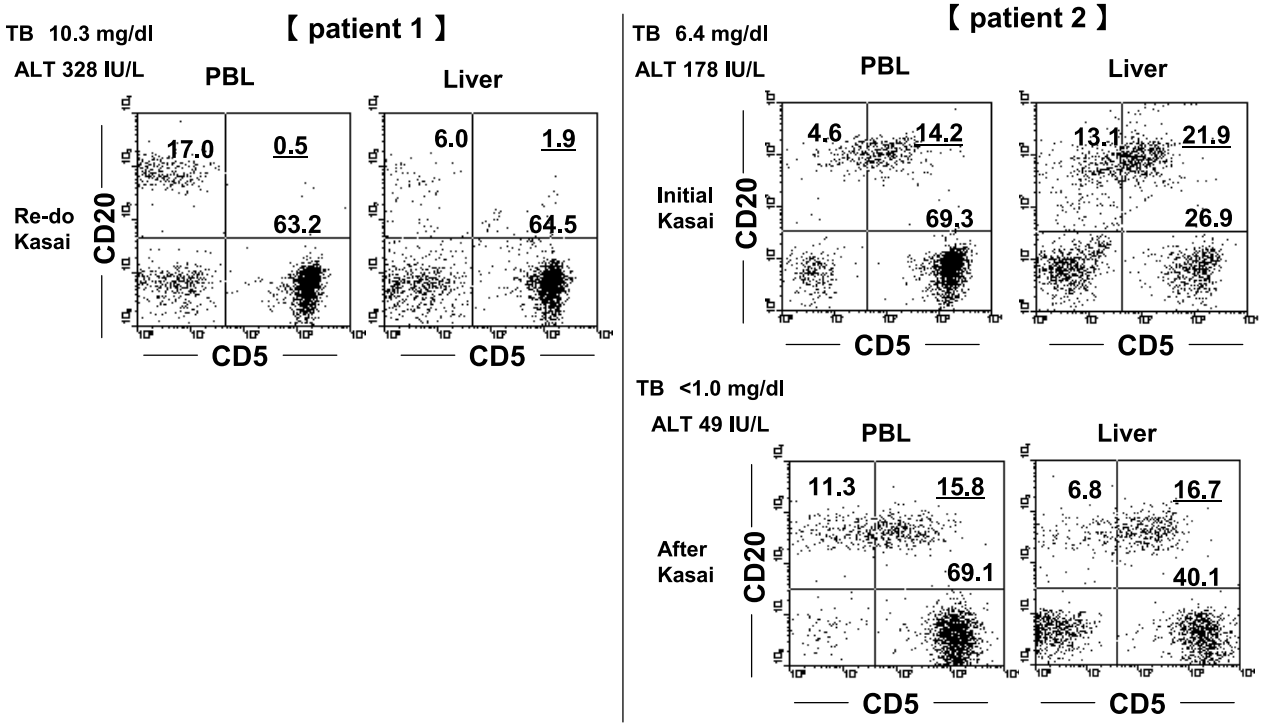

Fig.2. Changes in the $CD5^+$ B cell in the PBL and that in the liver in two patients with BA. The percentage of $CD5^+$ B cells in the PBL was not different from that in the liver for both patients. In patient 1, a marked reduction in the number of $CD5^+$ B cells was observed in the PBL and the liver at the re-do Kasai's operation. In patient 2, the percentage of these cells remains unchaged before and after Kasai's operation, regardless of the presence of jaundice.

adults, the relative size of both $CD8^+$ T cells and $CD56^+$ NK cells were found to be significantly higher in the liver than in the PBL, and these lymphocytes play an important role in liver immunity.[8] Therefore, the major subpopulations of lymphocytes in the liver are reported to differ phenotypically and functionally from those in PBL.[7,8,9,10,11]

In our study, considering $CD4^+$ and $CD8^+$T cells, $CD56^+$ NK cells, the relative sizes of these lymphocytes subpopulations in the liver were also different from those of PBL, and no significant correlations were observed between the presence of these cells in the liver and in PBL. Regarding these lymphocyte subpopulations, our results coincided with those of previous reports in adults.[7,8,9,10,11]

$CD5^+$ B cells are physically and functionally different from conventional B cells. Unlike conventional B cells that derive from human bone marrow, $CD5^+$ B cells originate from the fetal liver and fetal omentum in children. They are reported to dominate in the neonatal spleen and cord blood, and the percentage of such cells decreases with age. In normal children and adults, the mean percentages of $CD5^+$ B cells in PBL among total B cells are reportedly 18% and 5%, respectively.[6] In adults, $CD5^+$ B cells derive from peritoneum and spleen, and they constitute a tiny percentage of peripheral mononuclear cells. Adult human $CD5^+$ B cells produce IgM autoantibodies. These antibodies react with multiple antigens and are detectable in normal healthy individuals and in patients with autoimmune disorders. Therefore, these cells are considered to be related to autoimmune diseases.[6]

In the early infantile period, $CD5^+$ B cells are thought to play an important role in natural immunity. They react with multiple antigens by producing a low-affinity IgM, and these antibodies function as a first line defense in the early infantile period. They are thought to represent a bridge linking the innate and acquired immune responses.[6] Recently, $CD5^+$ B cells have been reported to be transformed into B/Macrophage and also to play a role in the cell-mediated immunity of the mouse.[12] In addition, $CD5^+$ B cells are also considered to affect the mucosal immune system in the intestines.[13] Therefore, $CD5^+$ B cells may be one of the important markers of systemic immunity in the early infantile period.

Regarding the role of $CD5^+$ B cells in the liver, these

cells have been reported to make a nodular/follicular aggregate in the liver in patients with HCV hepatitis, while T and NK cells are usually located generally in the portal tracts.[7,14] This finding suggests that the role of $CD5^+$ B cells in the liver is apparently different from those of T and NK cells. In adult patients with HCV hepatitis, an over expression of $CD5^+$ B cells in the PBL has been reported, and the expression level of $CD5^+$ B cells in PBL has been correlated with the histological activity index of the liver.[15,16] Furthermore, the progression of HCV hepatitis to cirrhosis has also been reported to be associated with a reduction in the number of hepatic $CD5^+$ B cells lymphoid aggregates.[14] On the other hand, according to the analysis of $CD5^+$ B cells in PBL in some kinds of liver disease, Yamada et al. reported that a significantly high level of $CD5^+$ B cells in chronic hepatitis and in liver cirrhosis — especially in hepatitis B surface antigen (HBsAg)-positive patients (hepatitis B virus carriers) — was observed. However, there was no significant difference between the percentages in normal controls and those in patients with acute hepatitis and primary biliary cirrhosis.[17]

These findings suggest that $CD5^+$ B cells play an important role in limiting disease progression; they are also thought to play a role in liver injury.[16] However, the degrees of expression of $CD5^+$ B cells in the liver may differ among various kinds of liver diseases.[17]

In our study, the relative size of the $CD5^+$ B cells in one BA patient showed no change before or after Kasai's operation, regardless of the presence of jaundice (patient 2). This finding may indicate that the expression of $CD5^+$ B remains stable up to a certain serum bilirubin level. However, a marked reduction in the number of $CD5^+$ B cells in a patient with a re-do Kasai's operation (patient 1) was observed for his age. The histological findings of this patient showed severe liver cirrhosis. Therefore, we speculate that the relative size of the $CD5^+$ B cells could become some kinds of indicator of liver damage in PCD. Though the precise reasons for these findings remain unclear, some damage to the stem cells of $CD5^+$ B cells due to an excess of the toxic metabolites of hepatic origin (bile acids, bilirubin, etc.) may have occurred with a progression of liver damage, as Socha et al. hypothesized.[3]

It is noteworthy that a significant correlation in the relative size of $CD5^+$ B cells between those in the PBL and in the liver was observed regardless of the type of disease. In addition, a good correlation in the $CD5^+$ B cell expression between the PBL and the liver was observed. We could not evaluate the correlation between the $CD5^+$ B cell expression and that of the MHC class II antigen or the IL-2R positive T cell because of the limited volume of the liver specimens. Although the detailed role of these cells in the normal infantile liver remains unknown and needs further studies, this finding suggests that we could obtain some information on the regional immunity of the liver by just an analysis of the PBL.

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