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INCREASED NUMBER OF HLA-DP POSITIVE T CELLS IN PATIENTS WITH AUTOIMMUNE HEPATITIS AND ITS ASSOCIATION WITH SERUM IMMUNOGLOBULIN LEVELS

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To determine whether activated T cells have a role in generating aberrant immune responses in autoimmune hepatitis (AIH), we assessed the prevalence of HLA-DP-positive T cells in relation to several serologic features. Our findings demonstrated a significantly higher number of such T cells in AIH than in hepatitis B surface antigen (HBs-Ag) positive chronic active hepatitis or healthy controls ($p<0.05$ in both studies), and that there was a strong correlation between the prevalence of HLA-DP T cells and serum immunoglobulin and IgG concentrations. Although HLA-DP antigen was primarily expressed on CD8 cells rather than CD4 cells (64. 0± 20.5% of CD8 cells, 37.3 ± 19.4% of CD4 cells), its association with serum immunoglobulin levels resulted from the elevation of HLA-DP CD4 cells, not HLA-DP CD8 cells. Thus, our findings suggest that HLA-DP antigens are expressed on various T cell subsets, one of which is the CD4 subset, and that these T cells play an important role in the generation of polyclonal hypergammaglobulinenia in AIH.

Introduction

Autoimmune hepatitis (AIH) is a chronic liver disease characterized by immunologic features similar to those of other autoimmune diseases, such as high concentrations of serum immunoglobulins and the presence of various autoantibodies. This disease tends to develop into liver cirrhosis in the very early period[1)2)]. As is true of other autoimmune diseases, neither its pathogenesis nor the proximate mechanisms of the immunologic abnormalities in this disease are yet unknown, however, several clinical features and immunologic parameters have been reported to be important in evaluating those immunologic abnormalities[3)~6)]. For example, HLA-DR antigen, normally expressed on limited lymphoid cells, is also expressed on many T cells in AIH patients to a degree commensurate with the severity of their hepatocellular injury[7)8)]. It was recently reported[9)~11)] that other HLA class II molecules, DP antigen, and DQ antigen also play unique roles in both normal immune responses and in the aberrant immune responses characteristic of autoimmune diseases.

In the present study, we attempted to elucidate the mechanisms of the aberrant immune response in AIH. We examined the prevalence of HLA-DP T cells in patients with this disease and compared it with their prevalence in healthy and disease controls. We also evaluated the function of these T cells in relation to serum immunoglobulin concentrations.

Patients and Methods

1. Patients

We assessed AIH (16 patients), type B chronic active hepatitis (B-CAH) (14 patients), and healthy

controls (15 persons). The mean ages of these groups were 59.1 years (range: 45 to 72) in AIH, 41.6 years (range: 16 to 75) in B-CAH and 42.9 years (range: 28 to 79) in the healthy controls. All of the subjects, including the controls, were female. The diagnosis of AIH was made on the basis of clinical and serologic features, which compatible with the diagnostic criteria in the previous report[12]. In brief, they were hypergammaglobulinemia (more than 2.0 g/ml), being positive for anti-nuclear antibody before initiation of corticosteroid therapy. All of these AIH patients had undergone liver biopsy at least once, and the histological picture of their liver lesions was found to be compatible with a diagnosis of chronic active hepatitis, and simultaneous liver cirrhosis observed in five of them. These AIH patients were carefully selected on the bases of having neither hepatitis B virus (HBV) nor hepatitis C virus (HCV) related markers in their sera. HBV markers were tested for with an EIA kit, (Abbott Laboratories, North Chicago, IL, USA), and the 2nd generation anti-HCV antibody assay was performed using an anti-HCV EIA kit (Ortho Inc., Raritan, NJ, USA). At the time of their study, 5 of the patients had not received any active medication, while the others had received corticosteroid (3 patients: 20 mg/day, 2 patients: 10 mg/day, 1 patient: 7.5 mg/day and 5 patients: 5 mg/day).

2. Analysis of peripheral-blood T-cell subsets

We used two-color analysis with Fluorescence Activated Cell Sorter[13] (FACScan: Becton Dickinson Co., Mountain View, CA, USA) to analyze the peripheral blood T cell subsets. In brief, peripheral blood lymphocytes (PBLs) obtained from venous blood were prepared using Ficoll-Hypaque gradient. The PBLs were incubated with anti-HLA-DP antibody (Becton Dickinson Co., Mountain View, CA, USA) for 30 minutes (first incubation) and then washed thoroughly. Next, we incubated them for another 30 minutes with fluorescein isothiocyanate (FITC)-conjugated goat-anti-mouse IgG antibody. After blocking the residual binding sites with normal mouse serum for 15 minutes, we stained them with either phycoerythrin (PE)-labeled anti-CD3, anti-CD4 or anti-CD8, and then analyzed the positively stained cells using FACScan.

3. Statistical analysis

We used Student's t-test to statistically analyze the differences in the prevalence of various T cell subsets between the AIH and control groups, and Pearson's correlation, to assess the correlations between the prevalence of HLA-DP T cells and serum immunoglobulin concentrations. In both analyses, a p value less than 0.05 was considered significant.

Results

1. Prevalence of HLA-DP-positive T cells in AIH

The prevalence of HLA-DP T cells in AIH patients and in two control groups are shown in Table. HLA-DP T cells ranged from 12.7 to 84.9% (mean ± SD: 47.5 ± 18.7%) of the total T cell count in patients with AIH, 5.8 to 46.0% (25.7 ± 11.9%) in patients with B-CAH and 2.2 to 34.9% (18.6 ± 11.0%) in the healthy controls, and the differences between the values in the AIH group and the two control groups were statistically significant ($p<0.05$), whereas the difference between the B-CAH group and the healthy controls was not.

To more precisely analyze the HLA-DP T cells,

Table *Prevalance of HLA-DP-positive T cells in AIH*

	AIH[1]	B-CH[2]	Control[3]	p value	
				1) vs 2)	1) vs 3)
DP+CD3+/CD3+	47.5±18.7	25.7±11.9	18.6±11.0	<0.05	<0.05
DP+CD4+/CD4+	37.3±19.4	21.3±8.9	12.3±7.7	<0.05	<0.05
DP+CD8+/CD8+	64.0±20.5	37.5±15.1	27.8±18.2	<0.05	<0.05

1) : Autoimmune hepatitis, 2) : Chronic hepatitis type B, 3) : Healthy controls.

we further examined which T cells, CD4 or CD8, expressed HLA-DP antigens on their surface in each group. As shown in Table, both T cell subsets expressed HLA-DP antigen on their surface. The average proportion of HLA-DP T cells was 37.3 ± 19.4% of CD4 cells and 64.0 ± 20.5% of CD8 cells in patients with AIH, 21.3 ± 8.9% of CD4 cells and 37.5 ± 15.1% of CD8 cells in patients with B-CAH and 12.3 ± 7.7% of CD4 cells and 27.8 ± 18.2% of CD8 cells in normal controls.

2. Correlations between HLA-DP-positive T cells and serum immunoglobulin concentrations

To analyze the biological significance of these elevated HLA-DP T cells, we attempted to determine whether their prevalence was correlated

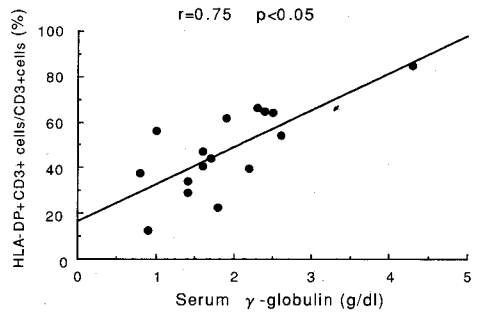

Fig. 1 Relation between the serum γ-globulin level and the ratio of HLA-DP CD3 cells to CD3 cells in AIH patients

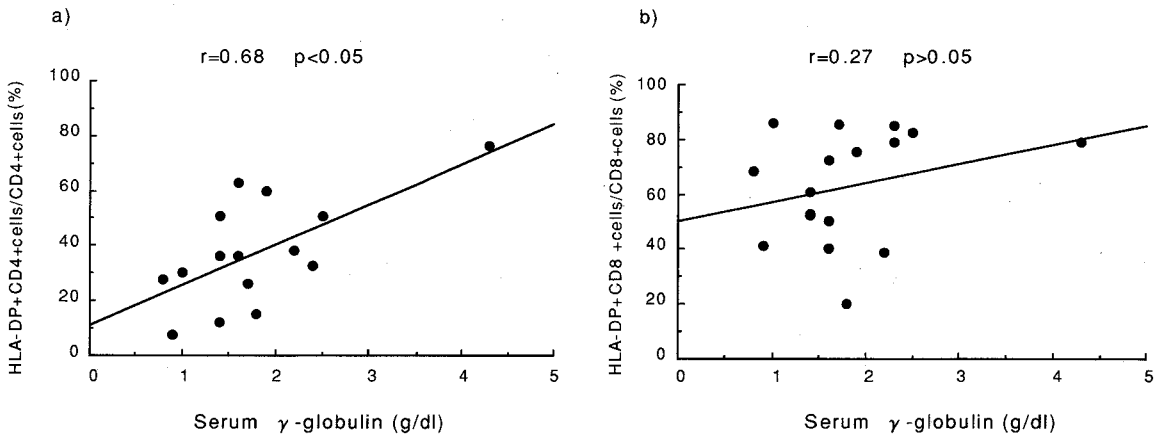

Fig. 2 Relation between the HLA-DP CD3 cells to CD3 cells and either the serum IgG level (a) or IgM level (b) in AIH patients

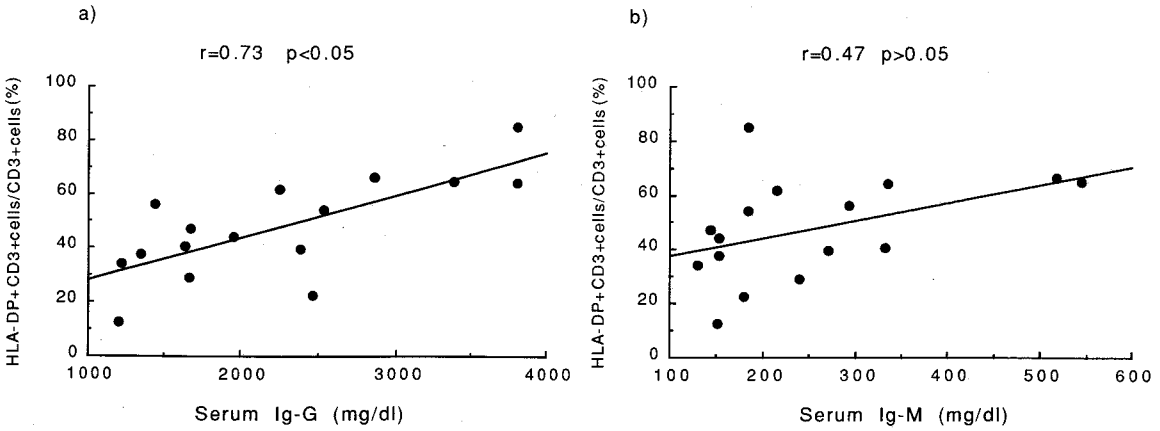

Fig. 3 Relation between the serum γ-globulin level and either the ratio of HLA-DP CD4 cells to CD4 cells (a) or the ratio of HLA-DP CD8 cells to CD8 cells (b) in AIH patients

with other immunologic parameters in AIH patients. The results showed a significant correlation between the prevalence of these cells and serum immunoglobulins ($p<0.05$) (shown in Fig. 1). To further investigate, we attempt to determine which of the immunoglobulin classes, IgG or IgM, was correlated with the number of HLA-DP T cells. The results shown in Figs. 2a and 2b, revealed that the prevalence of HLA-DP T cells was significantly correlated with the serum concentration of IgG not IgM.

As mentioned above (Table), HLA-DP antigen was expressed on both CD4 and CD8 cells. We conducted further studies to determine whether this association between the serum immunoglobulin concentration and HLA-DP T cells was primarily the result of the presence of HLA-DP CD4 or HLA-DP CD8 cells. As shown in Figs. 3a and 3b, there was a significant correlation between serum immunoglobulin concentrations and HLA-DP CD4 cells, but not HLA-DP CD8 cells. Thus, the correlation between HLA-DP T cells and serum immunoglobulin levels was due to the presence of HLA-DP CD4 cells, not CD8 cells.

Discussion

In patients with different autoimmune diseases, a majority of T cells bear several T cell activating markers not normally expressed on their surface of T cells in healthy individuals. HLA-DP antigens have also been recently repoerted[9] as activated T cell markers. Although the precise function of these HLA-DP T cells has not been well determined, they have been reported to have several important features for understanding the proximate mechanism of the immune response. For example, some of these T cells are closely associated with hypoproduction of interleukin-2 (IL-2) and HLA-DP antigen expression in the very early stage of activating T cells[10]. Regarding the relationship between these HLA-DP T cells and autoimmune diseases, it has been reported[10] that their numbers are increased in patients with systemic lupus erythematosus and that the prevalence of the cells correlates well with disease activity. As mentioned above, this T cell subset was reported to be related to the hypoproduction of IL-2, and it is thought to play an important part in the etiology of autoimmune diseases[10]. Based on these earlier reports, in our study, we focused on analyzing correlations between these HLA-DP T cells and serum immunoglobulin levels in patients with AIH. The results show that HLA-DP T cells are also elevated in these patients. Elevated numbers of HLA-DP T cells were found only in AIH, and not in B-CAH. Hence the elevation of this T cell subset might have some role in generating the aberrant immune responses. To analyze the role HLA-DP T cells, we tried to determine which T-cell subsets, CD4 or CD8, express HLA-DP antigen on their surface. The results show that it is expressed predominantly on CD8 cells ($64.0 \pm 20.5\%$) and that $37.3 \pm 19.4\%$ of CD4 cells were also positive for this antigen (Table). Thus, activated T cell markers, HLA-DP antigens, are expressed on various T cell subsets, suggesting that HLA-DP T cells may have multiple functions in AIH patients' aberrant immune responses. In fact, as shown in Figs. 3a and 3b, although HLA-DP antigens are mainly expressed on CD8 cells, the correlation with serum IgG concentrations resulted from the presence of HLA-DP CD4, not HLA-DP CD8 cells. Therefore, our results prompt us to think that HLA-DP CD4 T cells contribute to the hyperimmunity in these patients. Hence, HLA-DP T cells are belived to be related to hypoproduction of IL-2[10], we think that HLA-DP CD4 cells may belong to the Th2 type, which does not produce IL-2[14][15]. Regarding the function of HLA-DP CD8 cells, we found no inverse correlation between the prevalence of these cells and serum immunoglobulin concentrations, and so we believe that these HLA-DP CD8 cells may not act as suppressor T cells. Franco et al.[16] have reported the all CD8 cell clones derived from liver-infiltrated cells have cytotoxic activity. They did not determine whether their CD8 clones were HLA-DP. Based on these results, we can assume that these HLA-DP T cells act as cytotoxic T cells rather than as suppressor T cells. Further studies

are needed to answer these questions.

In the present study, we found that, as with other autoimmune diseases, patients with AIH have increased levels of HLA-DP T cells and that one of them, HLA-DP CD4 cells, may have some role in the hyperproduction of polyclonal immunoglobulin in these patients.

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自己免疫性肝炎患者における HLA-DP 陽性 T 細胞の増加と血清免疫グロブリン値との相関について

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自己免疫性肝炎における免疫異常を解析する目的で，活性化 T 細胞の指標である MHC クラス II 抗原である HLA-DP 抗原陽性 T 細胞の出現率を算定し，同時に測定した血清 γ グロブリン値，Ig-G，Ig-M 値との相関を検討し，その臨床的意義を検討した．方法は，自己免疫性肝炎患者，B 型慢性肝炎患者，および健常人の末梢血単核球を分離し抗 HLA-DP 抗体は間接法で FITC で染色し PE 標識した抗 CD3，CD4，CD8を加えて二重染色とし，flow cytometory で解析した．自己免疫性肝炎患者では，B 型慢性肝炎患者，健常者に比し有意に HLA-DP 抗原陽性 T 細胞の割合が増加していた．さらに DP 抗原陽性 T 細胞は，CD4，CD8抗原陽性の両者に認められたが，その比率は，CD8陽性 T 細胞が優位であった．さらに，同疾患患者において，血清中の γ グロブリンおよび Ig-G 値と，DP 抗原陽性 T 細胞の出現率との間には，強い相関関係が認められたが Ig-M 値との相関は認められなかった．これらの DP 抗原陽性 T 細胞と免疫グロブリン値の相関は，helper T 細胞機能をもつ CD4陽性 T 細胞の増加によるもので，killer-suprressor 機能をもつ CD8陽性 T 細胞の間には相関関係は認められなかった．

以上より，自己免疫性肝炎において，HLA-DP 抗原陽性細胞 CD4，CD8の両方の T 細胞に属するが，血清 γ グロブリン，特に血清 Ig-G の産生の増加には，CD4 DP T 細胞が，何らかの役割を果たしている可能性が示唆された．