Expression patterns of the C1q receptor (C1qRp) on the granulocytes and monocytes in peripheral blood mononuclear cells (PBMCs) from patients with liver diseases

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Abstract

Human C1qRp, a receptor for complement component 1, subcomponent q (C1q), was shown to be selectively expressed by cells of myeloid lineage, was originally reported to be involved in the C1q-mediated enhancement of phagocytosis. However, its expression patterns on the granulocytes and monocytes in human peripheral blood mononuclear cells (PBMCs) isolated from patients with various diseases are still not well understood. In this study, focusing on the clinical significance of the expression of this receptor in patients with chronic liver diseases, we demonstrated the expression patterns of C1qRp on the granulocytes and monocytes in PBMCs prepared from patients with some liver diseases, using the C1qRp monoclonal antibody (mAb) mNI-11 and flow cytometry. Analysis of the expression patterns (percentage of expression) of C1qRp on the granulocytes of patients with liver cirrhosis, chronic hepatitis B or C and hepatocellular carcinoma revealed a significantly reduced expression of this receptor in these patients as compared with that in normal healthy donors ($P<0.01$, $P<0.01$ and $P<0.01$, respectively). On the other hand, in relation to the expression of C1qRp on the peripheral blood monocytes in patients with liver diseases, while the expression was significantly decreased in the cells of patients with liver cirrhosis as compared with that of normal healthy donors ($P<0.01$), the expression levels in the cells of patients with chronic hepatitis B or C and hepatocellular carcinoma were not significantly different from those of normal healthy donors ($P=0.079$ and $P=0.534$, respectively). Taken together, these findings indicate that the expression of C1qRp on the granulocytes or monocytes was, in general, decreased in patients with liver diseases, but more significantly so in patients with liver cirrhosis.

Key words : C1qRp, granulocytes, liver diseases, monocytes, PBMCs

Introduction

Complement component 1, subcomponent q (C1q) is a key protein that is associated with the activities of the innate and adaptive immune systems. In addition to its physiological role in activating the classical complement pathway, C1q has been found to be capable of triggering a variety of cellular responses *in vitro* that may be mediated by specific receptors on the cell surface. Human C1qRp is a receptor for C1q, and was originally reported to be involved in the C1q-mediated enhancement of phagocytosis [1-3].

Human C1qRp has a molecular weight (m.w.) of about 90-100 kDa, and is a heavily *O*-glycosylated type I transmembrane protein consisting of unique C-type lectin domains [4-6], and exhibits strong homology (67-87% identity) to the rat and mouse C1qRp, also known as the AA4 antigen [7,8].

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Human C1qRp has been shown to be selectively expressed on granulocytes, monocytes and endothelial cells. Furthermore, mouse C1qRp also known as the AA4 antigen, was also found to be expressed in primitive hematopoietic stem cells[9], suggesting that this molecule may be involved in some important biological functions of cells, such as proliferation, differentiation, migration and adhesion.

The regulation of C1qRp expression has been investigated in a variety cells, in particular, in granulocytes, and it has been revealed that the inflammatory peptide FMLP rapidly upregulates the expression of this molecule[10]. However, the expression patterns (expression levels) of C1qRp on the granulocytes and monocytes in the peripheral blood mononuclear cells (PBMCs) prepared from patients with various diseases are still unknown.

In this study, focusing on chronic liver diseases, we investigated the expression patterns of C1qRp on the granulocytes and monocytes in PBMCs prepared from patients with some liver diseases, using the C1qRp monoclonal antibody (mAb) (mNI-11)[11] and flow cytometry, to define its clinical significance.

Materials and Methods

Donors

We examined preparations of human peripheral blood mononuclear cells (PBMCs) derived from 20 normal healthy donors, 8 patients with chronic hepatitis B or C, 12 patients with liver cirrhosis, and 16 patients with hepatocellular carcinoma. The diagnosis of the liver disease in each patient was established by histopathological examination. Informed consent was obtained from all of the patients and the normal healthy donors prior to their participation in the study.

Reagents

A C1qRp monoclonal antibody (mAb), mNI-11 (mouse IgG1)[11], was prepared in our laboratory. A fluorescence isothiocyanate (FITC)-conjugated goat anti-mouse IgG antibody was purchased from Cosmo Bio Co. (Tokyo Japan).

Preparation of human PBMCs

The human PBMCs from all the donors were prepared by the Ficoll-Paque (Pharmacia, Uppsala) density sedimentation method, as previously described[12].

Flow cytometry

The expression patterns of C1qRp on the PBMCs (granulocytes and monocytes) from all the donors were determined using an indirect immunofluorescence method. The PBMCs were first incubated with 20% normal human serum (NHS) to block the Fc receptor (FcR) for IgG, and then the cells were incubated with an optimum concentration of C1qRp mAb (mNI-11) for 40 min at 4℃. After washing twice with phosphate-buffered saline (PBS), the cells were incubated with an FITC-conjugated goat anti-mouse IgG antibody for 20 min at 4°C. They were finally washed with PBS and resuspended in cold PBS, then gated with granulocyte and monocyte populations under the exclusion of cellular debris and aggregates. The percentage of positively stained cells for mNI-11 in each population was analyzed by the use of a fluorescence-activated cell sorter (FACScan; Becton Dickinson, Tokyo, Japan). The number of positive cells was then obtained from logarithmic histograms of fluorescence intensities. The superior level of fluorescence intensity obtained for the isotype-matched normal mouse IgG (negative control) was considered as the limit of background fluorescence and the threshold of positivity for the mNI-11. In each sample, at least 1000 cells were analyzed. All specimens were analyzed in a masked manner, in that the examiner did not know the clinical history of patients.

Statistical analysis

The results shown were expressed as the means ± standard deviation (SD). The statistical analysis was performed using Mann-Whitney's *U*-test. Differences with a P value of less than 0.05 were considered to be significant.

Results and Discussion

In this study, we investigated the expression patterns of C1qRp on the granulocytes and monocytes in PBMCs isolated from normal healthy donors and patients with liver diseases, using a C1qRp mAb (mNI-11) and flow

cytometry. Figs. 1 and 2 show the typical histograms of C1qRp expression on the granulocytes and monocytes. The results are summarized in Figs.3 and 4. As shown in Fig. 3, the expression patterns (percentage of expression) of C1qRp on the granulocytes of patients with liver cirrhosis, chronic hepatitis B or C and hepatocellular carcinoma were significantly decreased as compared with that of the normal healthy donors ($P<0.01$, respectively). Fig. 4 shows that while the percentage of C1qRp expression on the monocytes of patients with liver cirrhosis was significantly decreased as compared with that on the cells of normal healthy donors ($P<0.01$), that on the monocytes of patients with chronic hepatitis B or C and hepatocellular carcinoma did not differ significantly from that on the same cells of normal healthy donors ($P=0.079$ and $P=0.534$, respectively).

Human C1qRp has been shown to be selectively expressed on granulocytes, monocytes and endothelial cells [13]. The regulation of C1qRp expression on a variety cells, particularly granulocytes, has been investigated previously, and it has been reported that although freshly isolated granulocytes from PBMCs showed expression of C1qRp, the inflammatory peptide FMLP rapidly upregulated the expression of this molecule on the cells [10]. Furthermore, it was also reported that the protein kinase C activator, phorbol myristete acetate (PMA), strongly upregulated the C1qRp expression on several cultured cell lines [14]. However, for the first time, we have found decreased C1qRp expression on the granulocytes and monocytes of patients with liver diseases. These findings suggest that determination of the expression patterns of C1qRp, which has several biological functions, may be of clinical significance in patients with liver diseases.

The reduced expression of C1qRp on the granulocytes and monocytes of patients with liver diseases may be attributable to the induction of apoptosis by some chronic inflammatory processes. In fact, we obtained some interesting evidence of a significant decrease of C1qRp expression in a T cell leukemia cell line (Jurkat) treated with a CD95 (Fas) mAb or mitomycin-C as apoptosis-inducing substances (data not shown). In addition, it was reported that C1qRp-deficient mice lacked the mechanism to exclude apoptotic cells *in vivo* and that the C1qRp molecule may contribute to the removal of apoptotic cells [15]. The basis for this contention is not very clear at the present time, but we are conducting a detailed analysis of the mechanism(s) in the decrease of C1qRp expression using various PBMCs.

More recently, it was reported that the C1qRp expressed on the granulocytes and monocytes of PBMCs is susceptible to PMA, which causes shedding of this molecule, and that C1qRp shedding from the granulocytes and monocytes induced by PMA is accompanied by a decrease of C1qRp cell surface expression [16]. These data strongly suggest that alterations of C1qRp expression patterns may have some important physiological roles. Further analyses are needed to demonstrate the detailed mechanism(s), at the cellular and molecular levels, of the decrease of C1qRp expression on the granulocytes and monocytes of patients with liver diseases.

References

1 Tenner, A.J., Robinson, S.L., Ezekowitz, R.A.:Mannose binding protein (MBP) enhances mononuclear phagocyte function via a receptor that contains the 126,000 M(r) component of the C1q receptor. Immunity 3:485-493, 1995.

2 Nepomuceno, R.R., Tenner, A.J.:C1qRP, the C1q receptor that enhances phagocytosis, is detected specifically in human cells of myeloid lineage, endothelial cells, and platelets. J. Immunol. 160:1929-1935. 1998.

3 Tenner, A.J.:C1q receptors regulating specific functions of phagocytic cells. Immunobiology 199: 250-264, 1998.

4 Norsworthy, P.J., Taylor, P.R., Walport, M.J., et al.: Cloning of the mouse homolog of the 126-kDa human C1q/MBL/SP-A receptor, C1qR(p). Mamm. Genome 10:789-793, 1999.

5 Nepomuceno, R.R., Ruiz, S., Park, M., et al.:C1qRP is a heavily *O*-glycosylated cell surface protein

involved in the regulation of phagocytic activity. J. Immunol. 162:3583-3589, 1999.

6 Park, M., Tenner, A.J: Cell surface expression of C1qRP/CD93 is stabilized by *O*-glycosylation. J. Cell Physiol. 196:512-522, 2003.

7 Dean, Y.D., McGreal, E.P., Akatsu, H., et al.: Molecular and cellular properties of the rat AA4 antigen, a C-type lectin-like receptor with structural homology to thrombomodulin. J. Biol. Chem. 275:34382-34392, 2000.

8 Dean, Y.D., McGreal, E.P., Gasque, P: Endothelial cells, megakaryoblasts, platelets and alveolar epithelial cells express abundant levels of the mouse AA4 antigen, a C-type lectin-like receptor involved in homing activities and innate immune host defense. Eur. J. Immunol. 31: 1370-1381, 2001.

9 Danet, G.H., Luongo, J.L., Butler, G., et al.: C1qRp defines a new human stem cell population with hematopoietic and hepatic potential. Proc. Natl. Acad. Sci. U.S.A. 99:10441-10445, 2002.

10 Jack, R.M., Lowenstein, B.A., Nicholson-Weller, A. :Regulation of C1q receptor expression on human polymorphonuclear leukocytes. J. Immunol. 153:262-269, 1994.

11 Ikewaki, N., Inoko, H. :Development and characterization of a novel monoclonal antibody (mNI-11) that induces cell adhesion of the LPS-stimulated human monocyte-like cell line U937. J. Leukoc. Biol. 59: 697-708, 1996.

12 Ikewaki, N.: A novel human monoclonal antibody rapidly induces homotypic cell aggregation and promotes antibody-secreting activity by human B lymphoblastoid cell line IM-9. J. Clin. Immunol.17:127-139, 1997.

13 McGreal, E.P., Ikewaki, N., Akatsu, H., et al. :Human C1qRp is identical with CD93 and the mNI-11 antigen but does not bind C1q. J. Immunol. 168:5222-5232, 2002.

14 Ikewaki, N., Kulski, J.K., Inoko, H.: Regulation of CD93 cell surface expression by protein kinase C isoenzymes. Microbiol. Immunol. 50:93-103, 2006.

15 Norsworthy, P.J., Fossati-Jimack, L., Cortes-Hernandez, J., et al. : Murine CD93 (C1qRp) contributes to the removal of apoptotic cells *in vivo* but is not required for C1q-mediated enhancement of phagocytosis. J. Immunol. 172:3406-3414, 2004.

16 Bohlson, S.S., Silva, R., Fonseca, M.I., et al.:CD93 is rapidly shed from the surface of human myeloid cells and the soluble form is detected in human plasma. J. Immunol. 175:1239-1247, 2005.

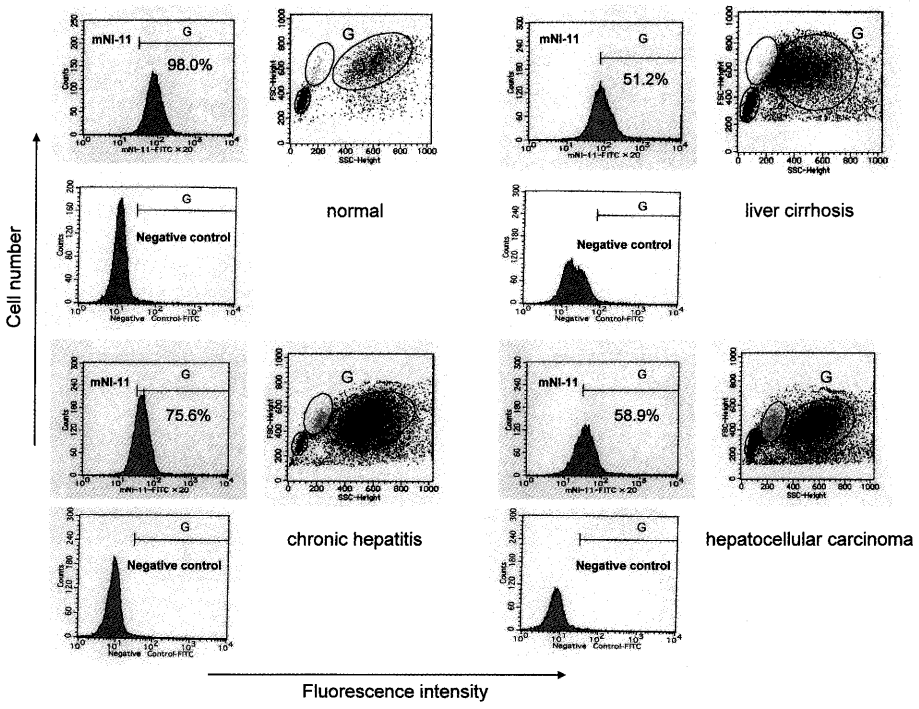

Figure 1. Typical histograms of the C1qRp expression patterns on the granulocytes of patients with liver diseases. The PBMCs were incubated with the C1qRp mAb (mNI-11) and FITC-conjugated goat anti-mouse IgG antibody. Negative controls were provided by incubation with isotype-matched normal mouse IgG. The cells were gated with granulocyte populations, and the percentage of positively stained cells for the C1qRp mAb (mNI-11) was determined using FACScan.

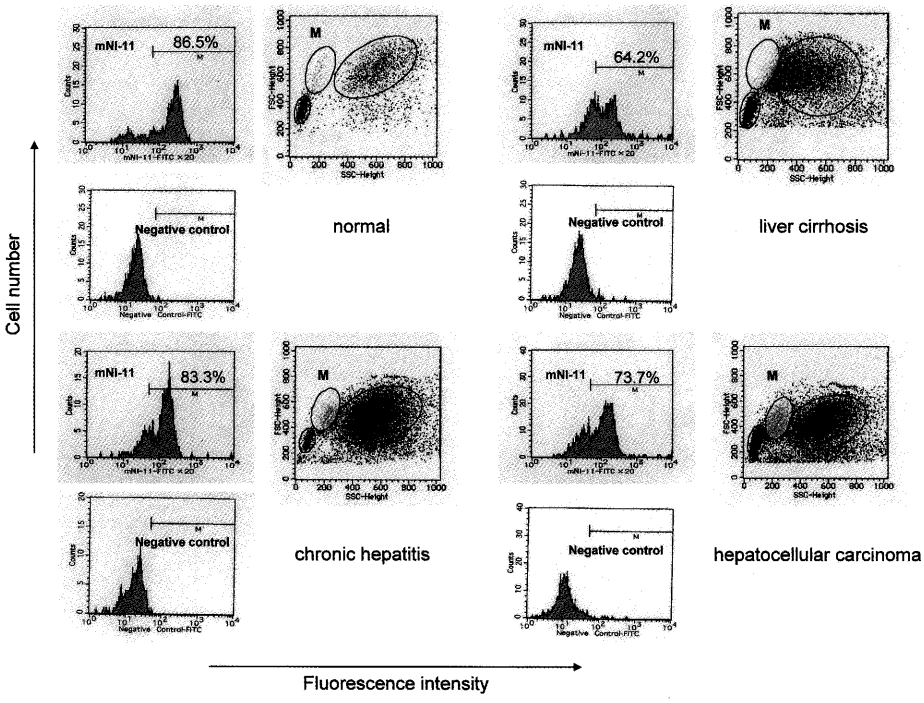

Figure 2. Typical histograms of the C1qRp expression patterns on the monocytes of patients with liver diseases. The PBMCs were incubated with the C1qRp mAb (mNI-11) and FITC-conjugated goat anti-mouse IgG antibody. Negative controls were provided by incubation with isotype-matched normal mouse IgG. The cells were gated with monocyte populations, and the percentage of positively stained cells for the C1qRp mAb (mNI-11) was determined using FACScan.

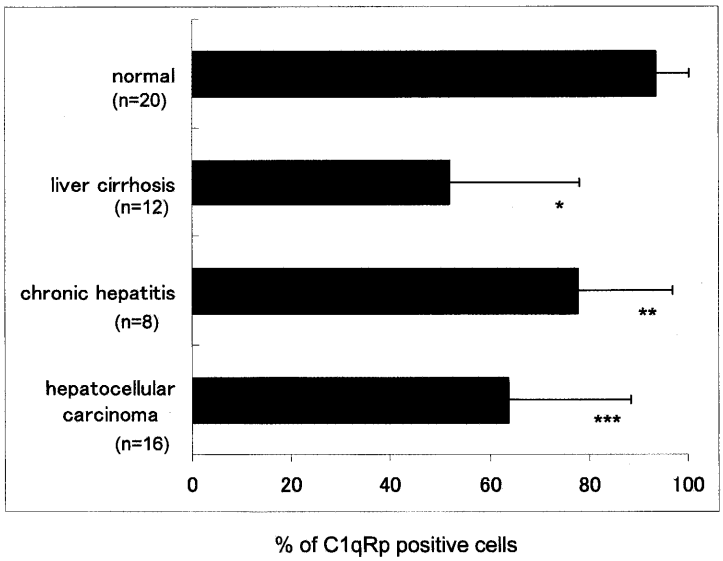

Figure 3. A summary of the results of the expression patterns of C1qRp on the granulocytes of patients with liver diseases. The PBMCs were incubated with the C1qRp mAb (mNI-11) and FITC-conjugated goat anti-mouse IgG antibody. Negative controls were provided by incubation with isotype-matched normal mouse IgG. The cells were gated with granulocyte populations, and the percentage of positively stained cells for the C1qRp mAb (mNI-11) was determined using FACScan. *P<0.01 (liver cirrhosis vs. normal), ** P<0.01 (chronic hepatitis vs. normal), ***P<0.01 (hepatocellular carcinoma vs. normal).

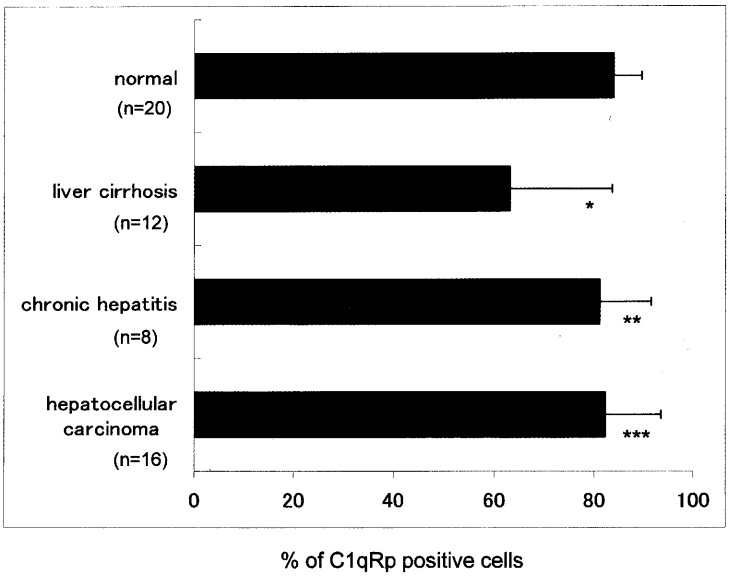

Figure 4. A summary of the results of the expression patterns of C1qRp on the monocytes of patients with liver diseases. The PBMCs were incubated with the C1qRp mAb (mNI-11) and FITC-conjugated goat anti-mouse IgG antibody. Negative controls were provided by incubation with isotype-matched normal mouse IgG. The cells were gated with monocyte populations, and the percentage of positively stained cells for the C1qRp mAb (mNI-11) was determined using FACScan. *P<0.01 (liver cirrhosis vs.normal), **P=0.079 (chronic hepatitis vs. normal), ***P=0.534 (hepatocellular carcinoma vs. normal).

肝臓疾患由来末梢血単核球（顆粒球および単球）表面上のC1qRpの発現パターン

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要　約

肝臓疾患由来末梢血単核球（顆粒球および単球）表面上の補体レセプター（C1qRp）の発現パターンを我々が開発したC1qRpモノクローナル抗体（mNI-11）とフローサイトメトリー法を用いて解析した。その結果、肝硬変、慢性肝炎、肝細胞癌患者由来の顆粒球表面上のC1qRpの発現は、健常人に比べて有意に低下していた（$P < 0.01$）。また、肝硬変患者由来単球表面上のC1qRpの発現も健常人に比べて有意に低下していた（$P < 0.01$）。しかしながら、慢性肝炎、肝細胞癌患者由来の単球表面上のC1qRpの発現は健常人と比べて有意な差は認められなかった（$P = 0.079$ および$P = 0.534$）。以上の結果は、肝臓疾患由来の顆粒球および単球の免疫学的な機能を解析する上で非常に有益な情報を提供するものと考えられる。

キーワード：C1qRp、顆粒球、肝臓疾患、単球、末梢血単核球
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