ORIGINAL ARTICLE

Diminished Cellular Immune Response to Carbonic Anhydrase II in Patients with Sjögren’s Syndrome and Idiopathic Chronic Pancreatitis

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ABSTRACT

Context A serum antibody to carbonic anhydrase II has been reported in patients with Sjögren’s syndrome and idiopathic chronic pancreatitis.

Objective To evaluate cellular immune response to carbonic anhydrase II in patients with Sjögren’s syndrome and idiopathic chronic pancreatitis.

Patients Idiopathic chronic pancreatitis (n=23), Sjögren’s syndrome (n=12), alcoholic chronic pancreatitis (n=3) and normal controls (n=13).

Main outcome measures Proliferation assay of peripheral blood mononuclear cells.

Results Notable increased proliferation of the mononuclear cells upon stimulation with carbonic anhydrase II was observed in 2 patients with idiopathic chronic pancreatitis (9%) and 2 patients with Sjögren’s syndrome (17%) but not in patients with alcoholic chronic pancreatitis nor in normal controls. Among the four study groups, there was no significant difference in the prevalence rate of the positive proliferative responses (P=0.444).

Conclusion Carbonic anhydrase II may not be a major target antigen for the immunological process in the pathogenesis of Sjögren’s syndrome and idiopathic chronic pancreatitis. Serum antibody to carbonic anhydrase II may be detected in these patients as a consequence of the immune reaction against other antigens which mimic carbonic anhydrase II.

INTRODUCTION

Autoimmunity has been suggested as a possible etiologic factor of idiopathic chronic pancreatitis which is observed in 30 to 40% of patients with chronic pancreatitis (CP) [1]. Recently, autoimmune pancreatitis has been proposed as a new disease entity in patients with CP [2, 3, 4, 5]. Although various immunologic alterations have been described in patients with idiopathic CP [6, 7, 8, 9], the autoimmune mechanisms which contribute to the pathogenesis of autoimmune pancreatitis remain poorly understood. It has been well-documented that CP is occasionally observed as a complication in patients with Sjögren’s syndrome (SjS), primary biliary cirrhosis and sclerosing cholangitis [2, 3, 10, 11]. The novel concept of “autoimmune exocrinopathy” [12], “dry gland syndrome” [13], or “autoimmune epithelitis” [14] has been put forth to describe the above mentioned disease complex. It is hypothesized that these diseases may be...
manifestations of an autoimmune reaction against a common antigen which is expressed in the ductal epithelial cells of distinct exocrine organs. However, the molecular nature of the common antigen is still unclear. It has been reported that patients with SjS showed serum antibody reactive to carbonic anhydrase (CA) II [15, 16]. We, and other investigators, reported that the serum anti-CA II antibody was observed in patients with idiopathic CP [17, 18, 19] and autoimmune cholangitis [20]. Moreover, we reported that sialoadenitis was induced in mice carrying the H-2^s and H-2^u MHC haplotypes by intradermal immunization with human CA II [21]. Recently, it was reported that pancreatitis and sialoadenitis were successfully induced in nude mice by the adoptive transfer of spleen cells from neonatally thymectomized mice immunized with CA II [22]. These findings suggest that CA II may be one of the common target antigens on the epithelial cells of multiple exocrine organs and, hence, it may play some role in the pathogenesis of autoimmune exocrinopathy. This hypothesis prompted us to study whether patients with SjS and idiopathic CP exhibit a cellular immune response to CA II. The present study evaluated the proliferative response to CA II of peripheral blood mononuclear cells from patients with CP and SjS.

MATERIALS AND METHODS

Patients

Twelve patients with SjS and 26 patients with CP were involved in the present study. SjS was diagnosed according to previously defined criteria [23]. CP was diagnosed according to criteria set by the Japan Pancreas Society [24]. Patients with CP were divided into two groups, according to the etiology: 3 patients with alcoholic CP and 23 patients with idiopathic CP. In the patients with idiopathic CP, there was no obvious cause such as alcoholism, gallstones, or other identifiable etiologic factors. Among idiopathic CP patients, a positive anti-nuclear antibody was observed in four cases, an increased immunoglobulin level (greater than 2.0 g/dL) in two cases, and an increased IgG level (greater than 1,800 mg/dL) in four cases. On abdominal computed tomography, focal enlargement of the pancreas was detected in four cases. Endoscopic retrograde pancreatogram revealed occlusion or segmental stricture of the main pancreatic duct in three cases. No case showed either diffuse enlargement of the pancreas or diffuse narrowing of the main pancreatic duct. Thirteen normal controls were also enrolled.

Peripheral Blood Lymphocyte Proliferation Assay to CA II

Human CA II (electrophoretically purified from erythrocytes) was purchased from Sigma (St. Louis, MO, USA). Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by Ficoll Hypaque density-gradient centrifugation as previously reported [25]. PBMC were resuspended at 2x10^5 cells/well of 96-wells round bottom plates (Corning, New York, NY, USA) in RPMI-1640 medium containing 5% heat-inactivated human serum (blood type AB) and 200 U/mL of penicillin-G (Sigma, St. Louis, MO, USA). The cells were incubated at 37 °C for 5, 7 or 9 days at various concentrations (0, 20, 50, and 100 µg/mL) of CA II (200 µL of reaction volume). For the last 24 h in each culture period, the cells were pulsed with ³H-thymidine (1 µCi per well; NEN, Boston, MA, USA). Following cell collection with a multiple cell harvester, ³H-thymidine incorporation was quantified on a liquid scintillation counter. The assay was performed in pentacate. The minimum and maximum numbers of cpm were omitted and then an average of the mid-three cpm numbers were taken for an evaluation of the PBMC proliferative response to CA II. Based on the cpm numbers obtained, the delta-cpm and the stimulation index were calculated as follows: stimulation index = cpm incubated with CA II / cpm incubated without CA II, and delta-cpm = cpm incubated with CA II -
cpm incubated without CA II. Background trapping of $^3$H-thymidine in wells without the cells showed the cpm to be below 100 in all assays.

ETHICS

Informed consent for the study was obtained from all participants. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as revised in 1983, as reflected in a priori approval by the appropriate institutional review committee.

STATISTICS

The Bonferroni test and the chi-squared test were performed to compare mean values of the stimulation index and the prevalence of positive proliferation responses, respectively. Pearson’s correlation coefficient was used for an analysis of the correlation between the stimulation index and the delta-cpm. Statistical significance was evaluated by means of the StatView 4.0 software and was defined as a two-tailed P value of less than 0.05. All data were presented as mean±SD.

RESULTS

Optimal Condition for PBMC Proliferation Assay to CA II

The optimal antigen concentration and culture period for the PBMC proliferation assay with the stimulation of CA II were studied in several patients with SjS and idiopathic CP. Representative data in one patient with SjS (70 years old, female) are shown in Figure 1. For a 5-day culture period with three different CA II concentrations (20, 50, and 100 µg/mL), PBMC proliferation was scarcely observed. In contrast, after a 7-day culture period, PBMC showed dose-dependent proliferative responses to the stimulation of CA II. Interestingly, PBMC proliferation cultured with 100 µg/mL CA II for 9 days decreased as compared to the proliferation cultured with 100 µg/mL CA II for 7 days and cultured with 50 µg/mL CA II for 9 days. In other idiopathic CP and SjS patients, stimulation indices significantly increased in cultures which lasted for 7 days with 50 µg/mL CA II as compared to those of normal controls. Consequently, PBMC proliferation among patient groups and normal controls was studied in this assay condition (cultures which lasted for 7 days with 50 µg/mL CA II). In preliminary studies, several patients with SjS and idiopathic CP showed a low basal growth of PBMC without the stimulation of CA II (data not shown). This was probably due to a decreased autologous mixed lymphocyte reaction [26]. Thus, the present study excluded the patients who showed a basal growth of PBMC (without CA II antigen) lower than 400 cpm in a preliminary proliferation assay.

PBMC Proliferation to CA II

Figure 2 showed a fair correlation between the stimulation index and the delta-cpm in all patients and normal controls (r=0.701, P<0.001). All patients with SjS and idiopathic CP who showed positive results in the stimulation index (greater than 2.433 as described below) had a delta-cpm greater than 1,000. It is noteworthy that one patient with SjS (Figure 3) showed a high stimulation index (9.023) and a high delta-cpm (4,449).
The mean±SD of the stimulation index in normal controls was 1.329±0.552 (n=13). In the present study, positive results were arbitrarily defined when the stimulation index was higher than 2.433 corresponding to the mean +2SD of that in normal controls. Positive results were obtained in 2 of 23 patients with idiopathic CP (9%) and in 2 of 12 patients with SjS (17%) (Figure 3). All three patients with alcoholic CP and all 13 normal controls showed negative results. Among the four study groups, there was no significant difference in the prevalence rate of the positive proliferation responses (P=0.444). As compared to normal controls, the mean values of the stimulation index in patients with SjS (2.112±2.437, n=12; P=0.298), idiopathic CP (1.433±0.986, n=23; P=0.406), and alcoholic CP (1.214±0.200, n=3; P=0.732) were also not significant.

Nine patients with idiopathic CP were previously studied for serum antibodies to CA II [17], resulting in 3 patients with positive serum antibody and 6 with negative. It is of note that none of these patients showed positive proliferation responses to CA II. Both idiopathic CP patients with the positive reactions showed normal serum levels of gamma-globulin and immunoglobulin subclass, and negative results for anti-nuclear antibody and rheumatoid factor. One patient showed focal enlargement of the pancreatic head on computed tomography and occlusion of the main pancreatic duct in the pancreatic head by endoscopic retrograde pancreatogram. Biopsy specimens taken from the main pancreatic duct by endoscopy showed the infiltration of lymphocytes and plasma cells in the fibrous parenchyma of the pancreas.

DISCUSSION

Previous findings showed that patients with SjS and idiopathic CP have serum antibody to CA II [17, 18, 19]. The present study evaluated the cellular immune responses to CA II by PBMC proliferation assay.
Unexpectedly, the results showed no significant difference in mean values of $^3$H-thymidine incorporation of PBMC and also in the prevalence rate of the increased stimulation index in patients with SjS and idiopathic CP as compared to normal controls. This finding suggests that CA II may not be a major target antigen for the immunological process in the pathogenesis of SjS and idiopathic CP. If this is true, why did these patients show serum anti-CA II antibodies in the previous studies [15, 16, 17, 18, 19]? One possible explanation is “molecular mimicry”: serum anti-CA II antibody may be detected in patients with SjS and idiopathic CP as a consequence of cross-reactivity of the antibody against other antigens which mimic CA II. We previously reported the occurrence of serum anti-CA I and anti-CA II antibodies in patients with idiopathic CP [17]. Interestingly, the anti-CA I antibody was completely absorbed with CA I, but not with CA II. In contrast to CA II, which is widely expressed in human cells including ductal epithelial cells of the salivary glands and the pancreas, expression of CA I is mostly restricted to erythrocytes [27]. These findings suggest that patients with SjS and idiopathic CP primarily have an immune reaction against the third CA isozyme which is distinct from CA I and CA II. Subsequently, these patients may show serum antibodies which are cross-reactive with CA I or CA II.

CAs are zinc metalloenzymes which catalyze a reversible hydration of CO$_2$ [27]. Among the twelve CA isozymes which have been reported, CA IV was shown on the cell surface of cultured tumor cells derived from human pancreatic epithelium [28], and CA IX and XII were reported to localize on the basolateral plasma membrane of the ductal epithelial cells in the pancreas [29]. Thus, CA IV, IX and XII will be candidates for the primary target antigen which produced serum antibodies cross-reactive with CA I or CA II. Moreover, it is noteworthy that a small number of patients with SjS and idiopathic CP showed a significantly increased PBMC proliferation upon stimulation with CA II. This means that at least a small percentage of patients with both diseases possess lymphocytes reactive with CA II in peripheral blood, even though T-cell epitopes are not specific to the CA II molecule. Furthermore, the present study included no cases which fulfilled the diagnostic criteria of autoimmune pancreatitis proposed by the Japan Pancreas Society [30]. Thus, the findings in the present study cannot completely rule out a possible role of CA II in the pathogenesis of patients with autoimmune pancreatitis.

In summary, a small number of patients with SjS and idiopathic CP showed a proliferative response of PBMC to CA II. Together with our previous results described above [17], findings from the present study indicate that patients with SjS and idiopathic CP may have previously been sensitized by the third CA isozyme, along with CA I and CA II. It is possible that the third CA isozyme plays an important role as the common target antigen on the epithelial cells of exocrine glands in the pathogenesis of autoimmune exocrinopathy. To provide evidence for this hypothesis, a study for a serum antibody against recombinant proteins of CA IV, IX and XII is currently underway.

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Keywords Autoimmunity; Immunity, Cellular; Pancreas; Pancreatitis; Salivary Glands

Abbreviations CA: carbonic anhydrase; CP: chronic pancreatitis; PBMC: peripheral blood mononuclear cells; SjS: Sjögren’s syndrome

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