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α_1-ACID GLYCOPROTEIN EXPRESSION IN HUMAN LEUKOCYTES: Possible Correlation between α_1-Acid Glycoprotein and Inflammatory Cytokines in Rheumatoid Arthritis

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Abstract—α_1-Acid glycoprotein is an acute-phase reactant that becomes markedly elevated in serum during inflammation and has an immunosuppressive effect on lymphocyte functions. Patients with collagen diseases had significant increases of α_1-acid glycoprotein in their serum and on the surface of peripheral leukocytes compared with controls. The levels from patients with rheumatoid arthritis were higher than those from patients with systemic lupus erythematosus, mixed connective tissue disease, and Behçet's disease. In patients with rheumatoid arthritis, the value of serum α_1-acid glycoprotein correlated with disease activity. Among leukocyte subpopulations, monocytes showed more α_1-acid glycoprotein on their surface than polymorphonuclear leukocytes and lymphocytes. The cell surface expression of α_1-acid glycoprotein on cultured monocytes surface peaked after 48 h. Interleukin-1β and tumor necrosis factor-α stimulated the production of α_1-acid glycoprotein RNA message in peripheral blood mononuclear cells over 18–24 h during cell culture. The results show that serum α_1-acid glycoprotein reflects systemic disease activity in rheumatoid arthritis. Furthermore, monocytes may serve as a source of production of α_1-acid glycoprotein.

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INTRODUCTION

α_1-Acid glycoprotein (AGP) is a normal constituent of human plasma that increases in concentration in patients with acute inflammation and cancer, and thus is recognized as an acute-phase reactant (1, 2). Although AGP has been implicated in a number of physiological processes, including inhibition of platelet aggregation (3), immunosuppression of lymphocyte functions (4–7), and acceleration of human erythrocytes through micropores (8), its biological function(s) in human inflammatory diseases such as those involving collagen diseases remain(s) still unknown.

AGP is produced in the liver, and it is likely that this is the major source of this protein in man. Recently, it has been reported that mediators of inflammation could stimulate acute-phase protein synthesis in rat hepatoma cells, human hepatocytes, or human hepatoma cell lines (9–17). Besides the liver, AGP may also be produced in mononuclear leukocytes. Unlike AGP synthesized in liver, AGP in leukocytes is initially formed as a precursor molecule, cleaved, and released with a molecular weight similar to that of liver (18).

In the present paper we monitored the serum level of AGP and its expression on the surface of leukocytes in patients with collagen diseases. Next, we correlated the serum levels of AGP with disease activity in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Finally, we examined AGP mRNA expression in leukocytes in response to inflammatory mediators.

MATERIALS AND METHODS

Subjects. Patients entered into this study were seen at the First Department of Internal Medicine, Kagoshima University Hospital. The patient population included individuals with RA, SLE, mixed connective tissue disease (MCTD), and Behçet's disease, respectively, according to the criteria of the American College of Rheumatology (formerly the American Rheumatism Association) (19, 20) or of the Japanese Ministry of Health and Welfare.

Seventy-four serum samples were obtained from 42 RA patients [4 males, 38 females, age: 61.5 ± 12.2 years (mean ± *SD*), range 34–82 years]; 36 sera were obtained from 22 SLE patients (1 male, 21 females, age: 34.7 ± 11.8 years, range 18–56 years); 12 sera were obtained from 7 MCTD patients (1 male, 6 females, age: 38.6 ± 11.9 years, range 16–57 years); and 16 sera were obtained from 8 Behçet's disease patients (2 males, 6 females, age: 41.6 ± 13.9 years, range 24–64 years). The sera were collected and stored at −80°C before use. Sera were collected from patients both in remission and with active disease. Control sera from 34 healthy donors (18 males, 16 females, age: 38.2 ± 11.2 years, range 19–58 years) were provided.

Thirty-two patients with RA were receiving nonsteroidal antiinflammatory drugs (NSAIDs), two patients with RA were taking simple analgesic drugs, 14 patients with RA were taking disease-modifying antirheumatic drugs (DMARDs), six patients with RA were receiving corticosteroids, and two patients with RA were taking immunosuppressive drugs. Disease activity in the RA patients encompassed a wide range of joint inflammation and joint deformity. Active disease in joints was

diagnosed when at least two of three clinical features, e.g., tenderness, warmth, and swelling were present. For systemic disease activity, joint counts were performed using a Ritchie articular index (21). Active disease was defined as a joint count ≥ 5 and/or morning stiffness of >30 min duration. Radiographic severity of disease was defined according to the number of erosions in a recent radiograph of the hands and wrists.

Disease activity in SLE patients was graded at the time of blood sampling and was based on clinical categories proposed in detail elsewhere (22, 23). Briefly, involvement of one system without fever (e.g., arthralgia, arthritis, or rash) represented grade I activity; 18 serum samples from 10 patients met this criterion. Grade II included those patients who had fever and involvement of one system, or involvement of more than one system without fever; eight serum samples from five patients fit this category. Fever and involvement of at least two systems represented grade III activity; four serum samples from three patients were in this group. Six serum samples from four patients who had intercurrent infection. Infection was diagnosed by positive cultures in four instances (urinary tract infection: 2; pharyngitis: 1; pneumonia: 1) and by a therapeutic response that was strongly suggestive of infection in two cases. Many of the patients were receiving prednisolone at the time of the study.

The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were measured by the Westergren method (24) and the turbidometric method (25), respectively, in RA and SLE patients at the same time of blood sampling.

α_1-Acid Glycoprotein Measurement. Serum AGP concentration were measured by a single radial immunodiffusion assay according to a previously published method (26). Briefly, agarose gels containing goat anti-human AGP serum were prepared on a glass plates containing wells 2.5 mm in diameter. Serum samples (5 μl) were placed in each well. After incubation for 48 h at 22°C, the diameter of the precipitation ring was measured and correlated with a standard concentration curve.

Antibodies. The anti-human AGP mouse monoclonal antibody (IgG_{2a} type) was purchased from Cambridge Medical Diagnostics, Billerica, Massachusetts, and Zymed Lab., San Francisco, California. Goat-$F(ab')_2$ anti-mouse IgG affinity-purified antibody conjugated to fluorescein isothiocyanate (FITC) (TAGO, Burlingame, California) was prepared. The goat anti-human AGP polyclonal antibody was purchased from MBL Inc., Nagoya, Japan, and the goat anti-human immunosuppressive acidic protein (IAP) (27) polyclonal antibody was a gift from Dr. K. Tamura in Tohoku University School of Medicine, Japan. The monoclonal anti-CD11b (Coulter Immunology, Hialeah, Florida) was used as a leukocyte-specific antibody for positive control.

Cell Preparation and Cell Culture. Whole blood from patients and healthy donors was treated with anticoagulant (10 units/ml sodium heparin, Shimizu Pharmaceutical Co. Ltd., Shimizu, Japan). Mononuclear cells were separated by centrifugation according to the method of Böyum (28) on LSM (Ficoll–sodium diatrizoate) solution (Bionecs, Kensington, Maryland), and suspended in RPMI 1640 (Flow Laboratories, Rockville, Maryland) medium containing 10% fetal calf serum (FCS, Gibco, Grant Island, New York), 5×10^{-5} mol/liter of 2-mercaptoethanol and antibiotics. The cells were placed at a density of 2×10^6/ml in plastic culture dishes (Falcon 3002, Becton Dickinson, Oxnard, California) and cultured for varying time periods at 37°C in 5% CO_2. After culturing for 12 h, nonadherent cells were removed by three washes with RPMI 1640 medium. The remaining adherent cells were collected gently with a rubber cleaner and washed with cold RPMI 1640 medium. This cell preparation was 90% monocytes as judged by the phagocytosis of yeast cells and esterase staining. Polymorphonuclear leukocytes (PMNs) were separated by centrifugation on Mono-Poly resolving medium Ficoll-Hypaque (Flow Laboratories) and suspended in RPMI 1640 medium. The PMN-containing supernatant was then aspirated and contaminating red blood cells (RBCs) were lysed with hypotonic NaCl. This preparation contained more than 95% PMN after three washes with RPMI 1640 medium. Cell viability, assayed by trypan blue dye exclusion, was more than 95%.

Indirect Immunofluorescence Assay. Heparinized whole blood cells from patients with col-

lagen diseases or healthy donors, and cultured cells from healthy donors, were analyzed for reactivity with AGP antibody by an indirect immunofluorescence method using an Ortho Spectrum III laser flow cytometry system (Ortho Diagnostic System Inc., Westwood, Massachusetts). Normal diluted serum was used as a negative control antibody for background fluorescence. Anti-CD11b was employed as a positive control for leukocyte reactivity. Anti-human IgG_{2a} antibody was used for control for the anti-AGP antibody. All reagents were titrated against the positive control to obtain optimum reactivity. In brief, 1×10^6 cells were washed three times to remove residual AGP from cell surfaces and incubated with 5 μl of monoclonal anti-AGP antibody or negative control serum at 4°C for 30 min. The cells were rewashed three times and incubated with 15 μl of a 200-fold dilution of FITC-conjugated goat anti-mouse Ig at 4°C for 30 min. Then the cells were washed three times, and the fluorescence staining was recorded on the Ortho Spectrum III, which displayed the results of the analysis on a histogram. Thresholds were arbitrarily set so that positive cells for the negative control antibody would be less than 4%.

Inflammatory Mediators and Other Reagents. Interleukin (IL)-1β was provided by Ohtsuka Pharmaceutical Co. Ltd., Tokushima, Japan; IL-2 was from Takeda Pharmaceutical Industry Co. Ltd., Osaka, Japan; tumor necrosis factor-α (TNF-α) was purchased from Genzyme Co., Boston, Massachusetts; phytohemagglutinin (PHA) was also from Difco Laboratories, Inc., Detroit, Michigan; lipopolysaccharide (LPS) was from Sigma Chemical Co., St. Louis, Missouri; standard serum protein for single radial immunodiffusion assay, Protein-Standard-Serum for NOR-Partigen, was purchased from Hoechst, Behringwerke AG, Marburg, Germany.

Detection of AGP mRNA by Northern Blotting Analysis. Human leukocytes from healthy donors were stimulated by inflammatory mediators or agonists for varying culture time periods. Namely, leukocytes were separated into mononuclear cells and PMNs according to the method described above. These cells (2×10^6 cells in each experiment) were cultured for varying time periods (12, 18, 24, and 36 h) under the presence of either IL-1β (30 units/ml), or IL-2 (15 units/ml), or LPS (30 μg/ml), or TNF-α (20 units/ml). Then RNA was extracted from the cells at the designated time by acid guanidinium thiocyanate-phenol-chloroform method (29). The RNA was electrophoresed through agarose gel and transferred to nitrocellulose membrane as described elsewhere (30). The membrane was hybridized with ^{32}P-labeled AGP cDNA (HindIII-digested fragment) (31). Nonstimulated leukocytes were used in the control study. Similar amounts of RNA were loaded, which was confirmed on the same blot by probing for β-actin mRNA.

Statistical Method. Statistical calculations were performed with a programmable calculator by using preprogrammed statistics. The mean values are expressed with the standard deviations. The significant differences between the mean values were determined by Student's t test. The correlation coefficients were determined by linear regression analysis.

RESULTS

Levels of Serum AGP in Patients with Collagen Diseases. Serum AGP levels (mean $\pm$ *SD*) of patients with RA, SLE, MCTD, and Behçet's disease were 159.0 $\pm$ 61.1, 90.8 $\pm$ 51.0, 93.3 $\pm$ 38.1, 85.9 $\pm$ 24.9 mg/dl, respectively, which was significantly higher than that of healthy donors (67.9 $\pm$ 24.7 mg/dl) (Table 1). The values obtained from serum samples of patients with collagen diseases were markedly higher compared with control ($P < 0.05$), and the serum levels found in the RA patients were higher than those found in the

other collagen diseases ($P < 0.001$). These results indicate that serum AGP is elevated in patients with collagen diseases.

Relationship of AGP to Clinical Activity in RA and SLE. A variety of clinical features that reflect disease activity were investigated in the RA patients. We assessed the clinical activity based on the degree of joint involvement. Active joint disease was diagnosed if two of three signs, e.g., tenderness, warmth, or swelling, or if tender and/or swollen joints, morning stiffness, and radiographic severity of disease were present. As shown in Table 2, the group of patients with active RA (active, joint count ≥ 5, or morning stiffness >30 min) had a significantly higher AGP value than those patients with less inflam-

Table 1. Serum AGP Concentrations in Collagen Diseases[a]

Group	*N*	Mean (mg/dl)	*SD*
Healthy subjects	34	67.9	24.7
RA	74	159.0[b]	61.1
SLE	36	90.8[c]	51.0
MCTD	12	93.3[c]	38.1
Behçet's disease	16	85.9[c]	24.7

[a] See Materials and Methods for details. Significance levels were determined by Student's *t* test ($P \leq 0.05$ considered significant).
[b] $P < 0.001$ versus healthy subjects, SLE, MCTD, and Behçet's disease.
[c] $P < 0.05$ versus healthy subjects.

Table 2. Serum AGP, CRP Concentration, and ESR in Patients with RA[a]

Group	AGP (mg/dl) mean ± *SD*	CRP (mg/dl) mean ± *SD*	ESR (mm/h) mean ± *SD*
Joint activity			
Active ($N = 46$)	176.1 ± 65.3[b]	2.88 ± 2.84[d]	139.8 ± 23.4[e]
Inactive ($N = 28$)	130.9 ± 41.0	0.68 ± 0.47	124.7 ± 31.5
Joint count			
≥ 5 ($N = 44$)	179.8 ± 63.9[c]	2.97 ± 2.88[d]	137.6 ± 23.3
<5 ($N = 30$)	128.5 ± 41.5	0.68 ± 0.39	128.9 ± 32.3
Morning stiffness			
>30 min ($N = 37$)	179.2 ± 67.5[b]	3.25 ± 3.04[d]	138.0 ± 24.2
≤ 30 min ($N = 37$)	138.8 ± 46.7	0.80 ± 0.58	130.2 ± 30.2

[a] See Materials and Methods for details. Significance level were determined by Student's *t* test ($P \leq 0.05$ considered significant).
[b] $P < 0.005$ versus joint activity—inactive, morning stiffness ≤ 30 min.
[c] $P < 0.001$ versus joint count < 5.
[d] $P < 0.001$ versus joint activity—inactive, joint count < 5 and morning stiffness ≤ 30 min.
[e] $P < 0.05$ versus joint activity—inactive.

matory disease ($P < 0.05$). In addition, there was a striking correlation between clinical activity, AGP, and CRP levels. Although higher values in ESR from the clinically more active patient group were observed, there was no statistical difference between active and inactive patients in terms of ESR. These results indicate that in RA serum AGP level, like CRP, can serve as a marker of disease activity.

Increased disease activity failed to correlate significantly with increased levels of serum AGP in SLE patients (Table 3). There were no significant differences between patients with grade II and grade III diseases or between patients with grade I and grade III diseases. AGP levels in patients with SLE and infection were higher than those in patients with grade I and grade II disease ($P < 0.05$). CRP levels correlated with disease activity, however, ESR was enhanced only in SLE patients with infection. These results indicate that serum AGP does not correlate with disease activity in SLE.

AGP Expression on Peripheral Leukocytes in Patients with Collagen Diseases. We next wished to determine whether AGP expression on leukocytes correlated with active inflammatory disease. By using saturating amounts of monoclonal antibody against AGP, we analyzed the expression of AGP on the surface of leukocytes by flow cytometry (Figure 1). RA patients ($N = 27$) with active disease, defined as more than 30 min of morning stiffness, were selected, and SLE patients with varying degrees of active disease were included (three cases of grade I, six cases of grade II, and six cases of grade III). Additionally, MCTD patients ($N = 6$) whose ESR was more than 60 mm/h were studied. The surface expression of AGP was increased on mononuclear leukocytes from

Table 3. Serum AGP, CRP Concentration, and ESR in Patients with SLE[a]

Group	AGP (mg/dl) mean ± *SD*	CRP (mg/dl) mean ± *SD*	ESR (mm/h) mean ± *SD*
SLE grade I ($N = 18$)	76.1 ± 44.0[b]	0.44 ± 0.27[c]	50.2 ± 21.4[e]
SLE grade II ($N = 8$)	81.8 ± 33.5[b]	1.29 ± 0.45[c]	57.8 ± 22.6[e]
SLE grade III ($N = 4$)	103.3 ± 60.6	3.33 ± 0.64[d]	62.3 ± 18.3[e]
SLE + infection ($N = 6$)	138.7 ± 64.0	9.90 ± 3.28	121.2 ± 18.5

[a] See Materials and Methods for details. Significance levels were determined by Student's *t* test ($P \leq 0.05$ considered significant).
[b] $P < 0.05$ versus SLE + infection.
[c] $P < 0.001$ versus SLE grade III and SLE + infection.
[d] $P < 0.005$ versus SLE + infection.
[e] $P < 0.005$ versus SLE + infection.

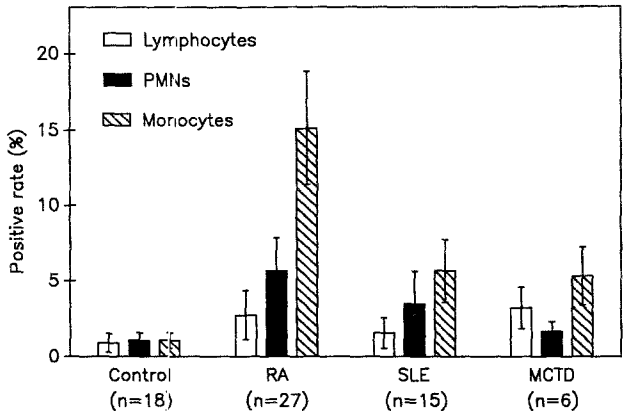

Fig. 1. AGP expression on leukocytes surface of subpopulations by flow cytometry. Leukocytes (1×10^6 cells) from patients with collagen diseases were incubated with monoclonal AGP antibody and with FITC-conjugated Ig, then measured by flow cytometry. Data represent mean ±SD. Percent of positive cells from mean fluorescent channel data was provided.

RA, SLE, and MCTD patients compared to control. The concentration of monocyte surface AGP was greatest in RA (15.2 ± 3.8%), followed by SLE (5.7 ± 2.1%), MCTD (5.3 ± 1.9%), and healthy donors (1.1 ± 0.5%). Statistically, the value of monocyte AGP from RA was significantly higher than those of the other groups ($P < 0.001$). Although leukocyte AGP was higher in all cell populations in RA compared to SLE and MCTD, the level of monocyte AGP was significantly greater compared to the other disease categories.

Time Course of AGP Expression on Monocyte Surface in Culture. To ascertain whether we could stimulate monocytes from healthy donors to express AGP, we monitored the changes of surface AGP expression on monocytes. AGP expression was measured by flow cytometry following stimulation by phytohemagglutinin (PHA) (Figure 2). AGP expression on the surface of monocytes stimulated with PHA increased over time. Maximal expression of AGP occurred at 48 h (18.4 ± 2.3%) compared to nonstimulated monocytes treated with buffer over the same time interval (4.0 ± 1.3%) ($P < 0.01$). These results indicate that AGP can appear on the monocyte surface following stimulation with PHA.

Detection of AGP mRNA. Since AGP expression on leukocytes might occur following AGP absorption from serum or could arise following leukocyte synthesis of AGP, we explored the possibility that AGP is produced in mononuclear leukocytes. We examined the production of AGP mRNA by mononuclear leukocytes. Mononuclear leukocytes were cultured in the presence of various stimulatory agents. The AGP mRNA was detected only in mononuclear cells harvested at 18 h following stimulation by IL-1β or by TNF-α at 24 h

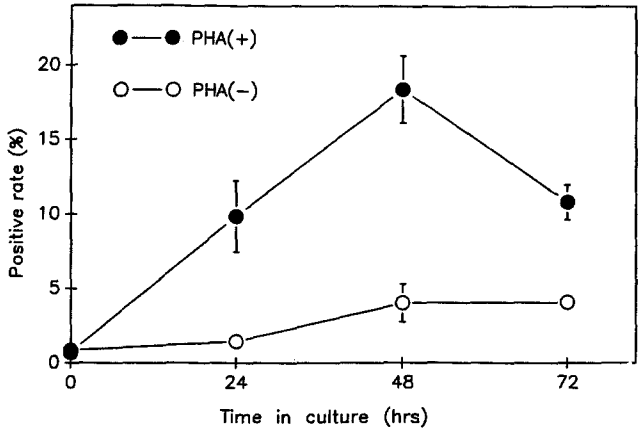

Fig. 2. Time course of AGP expression on monocytes surface in culture. Mononuclear cells (1×10^6 cells) from healthy donors were cultured in the presence (●—●) or absence (○—○) of 1% PHA. The cells were cultured for varying time periods, incubated with monoclonal AGP antibody and with FITC-conjugated Ig, then measured by flow cytometry. Percent of positive cells from mean fluorescent channel data was provided.

(Figure 3). On the other hand, neither IL-2 nor LPS stimulated AGP mRNA (data not shown). No AGP mRNA was detected in PMNs under the same condition (data not shown). Neither mononuclear cells nor PMNs produced detectable amounts of AGP mRNA when not stimulated. These results indicate that inflammatory mediators such as IL-1β and TNF-α can stimulate the production of AGP in mononuclear cells. Sufficient amounts of mRNA were available for analysis because identifiable β-actin mRNA was similar in stimulated and nonstimulated controls.

DISCUSSION

We found that AGP was expressed in peripheral mononuclear cells after stimulation by IL-1β and TNF-α (Figure 3). Gahmberg and Anderson (18) first reported that anti-AGP-antiserum and its $F(ab)_2$ fragments reacted with normal T and B lymphocytes, T lymphoblasts, PMNs, monocytes, and lymphoblastoid B-cell lines by immunofluorescence, and showed that AGP existed on the surface of lymphocytes. However, it was not clear in their work whether AGP on the surface of leukocytes resulted from passive absorption from serum or synthesis by these cells. We demonstrated the expression of AGP mRNA in leukocytes showing that de novo synthesis of AGP takes place in monocytes (Figures 2 and 3). The elevated serum concentration of AGP under various disease con-

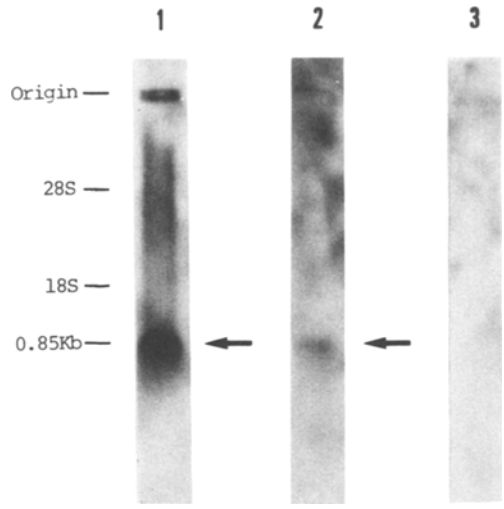

Fig. 3. AGP mRNA is detected in the mononuclear cells from healthy donors by northern blotting analysis. Leukocytes from healthy donors were separated into mononuclear cells and PMNs. These cells (2×10^6 cells/each experiment) were cultured up to 36 h (12, 18, 24, and 36 h) in the presence or absence of either IL-1β (30 units/ml), IL-2 (15 units/ml), LPS (30 μg/ml), or TNF-α (20 units/ml). At the designated time, RNA was extracted from the cells as described in Materials and Methods. The samples electrophoresed are: lane 1, 20 μg of RNA from IL-1β-stimulated mononuclear cells cultures at 2×10^6/ml for 18 h in RPMI 1640–10% FCS medium; lane 2, 20 μg of RNA from TNF-α-stimulated mononuclear cells cultured at 2×10^6/ml for 24 h in RPMI 1640–10% FCS medium; lane 3, 20 μg of RNA from nonstimulated mononuclear cells cultured at 2×10^6/ml for 18 h in RPMI 1640–10% FCS medium. Arrows indicate AGP mRNA. Positions of 28S and 18S ribosomal RNAs are indicated.

ditions (Table 1) (1, 2) has been somewhat difficult to explain by stimulation of liver synthesis alone. Although it is not possible to clarify in a quantitative way what proportion of AGP is derived from liver or leukocytes, it is conceivable that inflammatory states could stimulate leukocyte proliferation and increase AGP synthesis from leukocytes. Geiger et al. (12) reported that rat hepatoma cells showed an abrupt induction of AGP mRNA after IL-1 stimulation at around 12 h with a peak at 18 h in the presence of dexamethasone. Castell et al. (13) reported AGP synthesis appeared at about 20 h in cultured human hepatocytes stimulated with IL-6. Comparing our results (Figures 2 and 3) with their reports, induction of AGP mRNA after inflammatory stimulation in the monocytes appears to require a prolonged time in culture. Darlington et al. (9) and Mackiewicz et al. (11, 14) reported that monokines regulated acute-phase response. It may be possible to explain that the later phase induction of AGP mRNA is due to not only inflammatory mediators added into culture medium by itself but also an autocrine mechanism in mononuclear cells.

Of particular interest, we and others have found that AGP can serve as immunosuppressor of mitogen-included lymphocyte response (4–7). Our results suggested that inflammatory cytokine-stimulated monocytes produce and secrete AGP. This may lead to a higher concentration of AGP in inflammatory foci, which means AGP will regulate not only systemic but also local events. It is reasonable to assume that AGP plays an immunosuppressive role towards various antigens exposed by inflammatory injury, as occurs in collagen diseases. It is conceivable then that the elevated AGP levels in RA (Table 1) may serve to attenuate the immune response induced by various inflammatory stimuli. This possibility remains to be explored, since Denko and Wanek revealed an antiinflammatory action of AGP (32). It is of interest that AGP is highly expressed in decidua (33) and thus may play an important role in immunoregulation during pregnancy.

It has been reported that some inflammatory mediators can regulate the acute-phase response in hepatoma cell lines or hepatocytes in vitro (9–17). We employed IL-1β, IL-2, LPS, and TNF-α as stimulants. We found that significant expression of AGP mRNA occurred with IL-1β or TNF-α, but not with IL-2 and LPS (Figure 3). Then we extended the findings that cytokines can regulate the expression of AGP in hepatoma cells into mononuclear cells. We found that IL-1β induced AGP mRNA earlier than was seen with TNF-α; therefore, it is possible that in collagen diseases selective cytokines may stimulate mononuclear cells in vivo to express AGP. The differences in serum level and leukocyte surface expression of AGP among collagen diseases might be due to different mode of action of inflammatory mediators or different regulation of cytokine networks. We have previously reported that AGP was not related to IL-2 production from lymphocytes by mitogens (7). In this report we show that IL-2 is unlikely to be an effective stimulator for the expression of AGP message in leukocytes during inflammation.

Sarcione (34) first reported the biosynthesis of AGP in rat hepatocytes. AGP is produced in various kinds of tissue, for the most part in hepatocytes and hepatoma cell lines (9, 10, 12, 13, 34–36), hematopoietic cells (18), other malignant cells (37, 38), and other tissues (39, 40). According to the results of Gahmberg and Anderson (18) and Shibata et al. (41), AGP appeared to be produced in PMNs. This is in contrast to our present results, in which PMNs failed to increase AGP mRNA synthesis in response to a variety of stimuli. In support of our findings, Aozasa et al. (42) reported that reactive macrophages expressed an AGP-related protein known as immunosuppressive acidic protein in disorders such as histiocytosis X and neoplastic proliferation by immunohistochemical determination. These reports and our results (Figures 2 and 3) suggest that AGP is synthesized preferentially in monocytes rather than PMNs.

We also found that surface expression of AGP on peripheral blood monocytes reflected disease activity in patients with RA but not in SLE or

MCTD (Figure 1). Moreover, we found that AGP levels in the serum correlated best with disease activity in RA (Table 2). There was only a modest increase in serum AGP levels in either SLE, MCTD, or Behçet's disease compared to RA (Table 1). Only in SLE patients with intercurrent infections did AGP levels in the serum rise significantly (Table 3). Our findings are similar to reports by Pawlowski et al. (43) and Mackiewicz et al. (44), who found elevated levels of glycosylated AGP in serum of patients with SLE and intercurrent infections.

We have confirmed the induction of AGP mRNA in stimulated peripheral mononuclear cells. We suggest that AGP may play an important role in the inflammatory response involved in collagen diseases. The inflammatory mediators in patients with RA that could account for the elevated serum AGP in that disease as compared to the other collagen diseases remain to be determined. Further studies of interacting effects among AGP, inflammatory mediators, and leukocytes will provide a better pathophysiological understanding of the regulation of inflammation.

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