Age-related increase of autoantibodies to interleukin 1α in healthy Japanese blood donors

Yasukazu Ohmoto[*], Fumitaka Ogushi[†], Masahiro Muraguchi[*], Mayumi Yamakawa[‡] and Saburo Sone[†]

[*]*Otsuka Pharmaceutical Co., Kagasuno, Kawauchi-cho, Tokushima, Japan ;* [†]*Third Department of Internal Medicine, The University of Tokushima School of Medicine, Tokushima, Japan ; and* [‡]*Japan Red Cross Blood Center, Shomachi 3, Tokushima, Japan*

Abstract : Although autoantibodies to interleukin-1α (IL-1 α autoantibodies) are known to be present in sera of apparently healthy humans, their frequency of occurrence and significance are unclear. To determine the prevalence of detectable IL-1α autoantibodies in normal human blood, we screened the plasma of blood donors (6290 subjects : 3977 men and 2313 women, ages 16 to 64 yr) by a radioimmunoassay which we developed using a method that could detect over 5 ng/ml. Moreover, we investigated immunoglobulin class of IL-1α autoantibodies and also their function. IL-1α autoantibodies were detected in 14.6% of the 6290 donors. Their frequency was higher in males than females (16.6% vs.11.2%, p<0.01) and increased with age in both sexes. The proportion of subjects with a high IL-1α autoantibodies titers also increased with age. We showed that IL-1α autoantibodies were of the IgG class and that they had neutralizing function to IL-1α by receptor assay. Neutralizing activity was only shown in plasma with concentration of IL-1α autoantibodies, the level of which was over 1000 ng/ml. The affinity of the IL-1α autoantibodies in plasma was between 2.1×10^{-10} and 1.2×10^{-9} M (mean 6.4×10^{-10}M). Our results provide a basis for comparison with IL-1α autoantibodies prevalence between healthy states and disease states, and suggest that IL-1α autoantibodies may play a significant role in modulating the effects of excessive IL-1α at local site or in systemic regions. J. Med. Invest. 44 : 89-94, 1997

INTRODUCTION

Interleukin-1 (IL-1), first recognized as an endogenous pyrogen, has subsequently been found to be an important mediator of immune and inflammatory reactions (1-3). It is synthesized in response to infection, injury or antigenic challenge by a variety of cells including monocytes and macrophages (2), and exerts biologic effects on various target cells in normal and pathological conditions (1-3). IL-1 exists as two genomic forms, IL-1α and IL-1β, both of which are very similar in function. Although the precussor for IL-1β requires cleavage to reveal its IL-1 activity, the proIL-1α is fully active as a precussor and remains intracellulaly (1-3). IL-1α is thought to be involved in cell-mediated responses through cell-to-cell contact (1-3). Despite its host-defense role, IL-1 may cause tissue damage and exacerbate chronic inflammation.

Accordingly, its potent inflammatory effects are believed to be tightly regulated.

The actions of IL-1 are controlled in several ways, such as by regulation of its synthesis and by production of neutralizing factors including a soluble IL-1 receptor, and an IL-1 receptor antagonist (1, 3, 4, 7). Recently, antibodies against cytokines such as interferons, tumor necrosis factor, IL-6 and IL-10 have been detected in sera of patients with inflammatory and neoplastic diseases and in healthy subjects (8-12). Autoantibodies against IL-1α (IL-1α autoantibodies) have also been found in the sera of patients with inflammatory process and asymptomatic individuals (5, 6, 13-16). It is known that IL-1α auto-antibodies neutralize IL-1α specifically (14, 16, 17), while IL-1 receptor antagonist blocks the activity of both IL-1α and IL-1β (1, 3). However, the level of plasma IL-1α autoantibodies which neutralizes IL-1α activity was not clear. Thus, the role of IL-1α autoantibodies in blood remains to be defined. However, the reported prevalence of IL-1α autoantibodies in the blood of healthy subjects was inconsistent (6, 13-16). Their frequency of occurrence

in normal human blood needs to be established, because fresh plasma is transfused into patients as an element of blood, and also because such data sheds light on the roles of IL-1α autoantibodies in healthy and diseased subjects. We developed sensitive radioimmunoassay (RIA) for IL-1α autoantibodies, and measured IL-1α autoantibodies levels in plasma from a large number of healthy blood donors, considering overall prevalence, sex and age. Furthermore, we characterized the immunoglobulin class of IL-1α autoantibodies as well as elucidating the level of plasma IL-1α autoantibodies to neutralize IL-1α activity.

MATERIALS AND METHODS

Study Population

Plasma was obtained from donor blood at the Tokushima Blood Transfusion Center in Japan. Samples were obtained from 6290 healthy donors (2313 women and 3977 men between 16 yr and 64 yr). Samples were separated into donor groups I-VI, respectively aged 16-19, 20-29, 30-39, 40-49, 50-59, and 60-64 yr. Age and sex distributions of donors are shown in Table 1.

Table 1. Distributions by age and sex of blood donors

Group	Age (ys)	Males	Females	Total
I	16-19	711	546	1257
II	20-29	844	446	1290
III	30-39	1032	484	1516
IV	40-49	947	479	1426
V	50-59	389	300	684
VI	60-64	54	58	112
Total		3977	2313	6290

Measurement of IL-1α autoantibodies

Human recombinant IL-1α (rIL-1α) was prepared as described previously (18). IL-1α autoantibodies in plasma were measured by a radioimmunoassay (RIA). Briefly, duplicate 50 μl samples of plasma or standard dilutions of monoclonal antibodies to IL-1α (ANOC 301, purity >95%), ranging from 5 to 640 ng/ml, were mixed with ^{125}I-rIL-1α. Each sample was mixed with 0.1% bovine serum albumin/ phosphate buffer saline (BSA/PBS) containing 5 mM ethylenediaminetetraacetic acid (EDTA)/0.05% NaN₃, and each standard dilution of rIL-1α was mixed with 0.1% BSA/PBS containing 5 mM EDTA/0.05% NaN₃ and carrier buffer (2% bovine γ globulin). After antigen-antibody binding had reached equilibrium by overnight incubation at room temperature, each sample was mixed with 25% polyethylene glycol (MW 6000) and incubated for 1hr. Then, the tubes were centrifuged at 3000 rpm for 15 min, and the radioactivity of the precipitates were counted for 1 min in an automated γ-spectrometer. The IL-1α autoantibodies titer was determined from the standard dilution curve using IL-1α monoclonal antibody (Fig.1). Plasma was considered IL-1α autoantibodies-positive at levels over 5 ng/ml. Autoantibodies for IL-1β (IL-1β autoantibodies) in the plasma were measured by the same
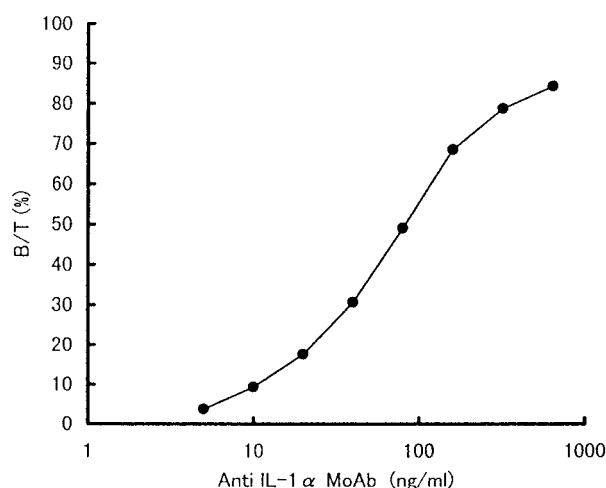

Fig.1. Standard curve of IL-1α autoantibodies by RIA. The standard dilution of IL-1α monoclonal antibody (ANOC 301), ranging from 5 to 640 ng/ml, were mixed with ^{125}I-labeled rhIL-1α. The mixture was centrifuged and precipitates were counted by γ-spectrometer. B/T (%) : the percentage of bound ^{125}I-labeled IL-1α (B)/total ^{125}I-labeled IL-1α. (T).

method as described for IL-1α autoantibodies, substituting IL-1α by IL-1β. The sensitivity of this assay for IL-1β autoantibodies was 5 ng/ml.

Characterization of IL-1α autoantibodies in plasma

To characterized IL-1α autoantibodies, we used plasma containing a high IL-1α autoantibodies level and investigated immunoglobulin class of IL-1α autoantibodies. The plasma was applied to a protein A agarose column. After washing with the binding buffer (50 mM sodium borate with 3M NaCl, pH 9.0), the bound material was eluted with elution buffer (100 mM glycine, pH 2.5) and collected in 2 ml fraction. The IL-1α autoantibodies level in each fraction was measured by RIA. The plasma IL-1α autoantibodies was also fractionated by gel filtration using Sepharose 6B column. Plasma was applied to a column preequilibrated with 50 mM Tris-HCl containing 0.5 M NaCl and 5 mM EDTA (pH 8.0) and eluted with the same buffer. The elute was collected in 3 ml fractions and the IL-1α autoantibodies concentration of each fraction was measured by RIA.

IL-1α receptor assay

Neutralizing activity of IL-1α autoantibodies in plasma was examined by IL-1 receptor assay as described previously (19). ^{125}I-labeled IL-1α was prepared by the same way as described in the method of RIA. The specific activity of ^{125}I-labeled IL-1α was 130-150 μCi/g. BALB/3T3 fibroblasts (the American Type Culture Collection) were maintained in a Dulbecco's minimum essential medium (Nissui Pharm. Co. Ltd., Tokyo, Japan) containing 10% fetal calf serum (Hyclone Inc., Utah). 3T3 fibroblasts (1 X 10⁶) were incubated with 500 μl of culture medium containing ^{125}I-labeled IL-1α (10⁵cpm) and 10% sera from the patients in 12-well plates (Corning Glass Works, NY) for 2 hr at 4℃. Nonspecific binding was determined in the presence of 1 μg/ml of unlabeled IL-1α. The cell monolayers were then rinsed 3 times with 1 ml of PBS

then solublized with 1 ml of a mixture of 1% SDS and 0.2 N NaOH. The radioactivity was counted in a γ-counter.

Equilibrium binding assay

To determine the affinity of IL-1α autoantibodies in plasma for human IL-1α, the dissociation constant (Kd) of IL-1α autoantibodies/IL-1α complexes was measured. The plasma IL-1α was incubated with increasing amount of ^{125}I-IL-1α in the final volume of 200 μl in RPMI 1640, 1% BSA. After 4 hr incubation at 4℃, IL-1α autoantibodies/IL-1α complexes were precipitated with protein G-sephadex and the radioactivity of the precipitate was measured by a γ counter. Ratio (B/F) of specific bound ^{125}I-IL-1α (B) vs free ^{125}I-IL-1α (F) were calculated, then plotted vs specific bound ^{125}I-IL-1α amounts calculated according to the IL-1α specific activity. According to Scatchard, the Kd value was determined by the slope of the obtained straight line (Kd=-1/slope) and expressed in concentration according to the reaction volume.

Statistical Analysis

Statistical analysis was performed by the $\chi 2$ test, and values were considered significantly different at $p<0.05$.

RESULTS

Standard curve of IL-1α autoantibodies in RIA

The standard curve of IL-1α autoantibodies, as measured by RIA, is shown in Fig.1. From this standard curve, the IL-1α autoantibodies concentration in plasma of normal healthy subjects was calculated, and ranged from 5 to 640 ng/ml by this assay.

Prevalence of IL-1α and β autoantibodies in normal human plasma

Prevalence of IL-1α autoantibodies in plasma are shown in Table 2. IL-1α autoantibodies was detected in 917 (14.6%) of samples. Its prevalence was higher in males than in females in general (16.6% vs 11.2%, p<0.01) and in groups II and IV (p<0.01). As shown in Table 2, the prevalence of IL-1α autoantibodies increased with age : in groups I and II, it was less than those in groups III, IV, V and VI (p<0.01), but there were no significant differences in the groups over 40 yr. In a parallel study, we measured IL-1β autoantibodies in plasma from about 500 normal subjects. We could not detect IL-1β autoantibodies in plasma from any subjects (data not shown).

Distribution of subjects with high titers of IL-1α autoantibodies in the blood

The proportions of subjects with an IL-1α autoantibodies level exceeding 1000 ng/ml among the antibody-positive individuals are shown in Figure 2. The percentage of subjects with a high titer of IL-1α autoantibodies increased steadily with age, being 2 (2.9%) in group I, 8 (6.1%) in group II, 27 (10.9%) in group III, 41 (13.2%) in group IV, 27 (19.7%) in group V and 7 (29.2%) in group VI.
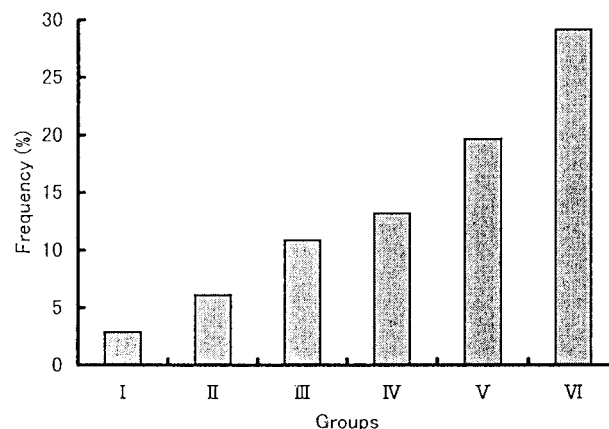

Fig. 2. Distribution of normal individuals with high plasma titers of IL-1α autoantibodies. Bars show percentages of subjects with IL-1α autoantibody levels of over 1000 ng/ml among the antibody-positive cases.

Characterization of plasma IL-1α autoantibodies

To characterize IL-1α autoantibodies, we used plasma containing a high IL-1α autoantibodies level. The sample was applied to a protein A agarose column. After washing with the binding buffer, we obtained the IgG fraction using an elution buffer. RIA showed that the high titer of IL-1α autoantibodies was presented only in the IgG fraction (Fig.3). Furthermore, the plasma was fractionated by gel filtration resulting in its elution with an apparent molecular weight of 100,000-200,000, IL-1α autoantibodies corresponded to that of IgG (Fig.4). The affinity of the IL-1 α autoantibodies in several samples, examined by the Scatchard analysis, was between 2.1×10^{-10} and 1.2×10^{-9} M (Table 3).

Neutralizing activity of IL-1α autoantibodies

First, we examined whether neutralizing activity of IL-1α autoantibodies was estimated by IL-1α receptor assay using BALB/3T3 fibroblasts. Standard of IL-1 receptor assay was performed using monoclonal antibody to IL-1α (ANOC 301). As shown in Fig.5, monoclonal antibody (ANOC 301) showed neutralizing activity in a dose dependent manner. Next, to examine the neutralizing activity of IL-1α autoantibodies in plasma, we performed an IL-1α receptor assay. Plasma from subjects with a concentration of IL-1α auto-

Table 2. Incidences of antibodies to IL-1α in blood donors

	Group I 16-19 ys	Group II 20-29 ys	Group III 30-39 ys	Group IV 40-49 ys	Group V 50-59 ys	Group VI 60-64 ys	Total
Males	6.0	12.4[a]	17.3	24.6[a]	22.6	22.2	16.6[a]
Females	4.8	5.8	13.2	14.5	19.0	19.0	11.2
Total	5.5[b]	10.2[c]	16.0	21.5	21.0	20.5	14.6

Date show percentages of positive cases in males and females in each in group.
   a) p<0.01, significance difference between males and females.
   b) p<0.001, significance difference between Group I and Groups II-VI.
   c) p<0.001, significance difference between Group II and Groups III-IV.

Table 3. Affinity constant of IL-1α autoantibodies in high titers subjects

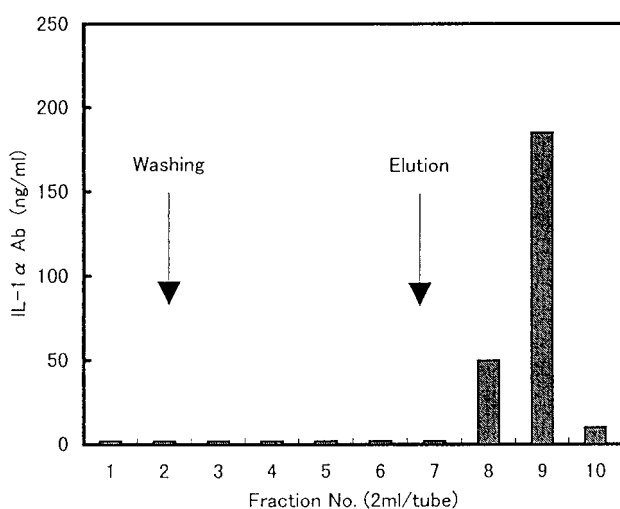| Donor	Affinity constant ($\times 10^{-10}$M)
1	7.41
2	6.21
3	5.03
4	6.95
5	3.10
6	11.71
7	2.07
8	7.17
9	7.56
10	3.83
11	5.88
12	10.30
Average	6.4

Fig. 3. Fractionation of plasma IL-1α autoantibodies by a protein A agarose column. Plasma with high IL-1α autoantibody levels were diluted and applied to the protein A column. After washing with the binding buffer, protein was eluted in 2 ml fractions by an elution buffer. Then, the IL-1α autoantibodies level in each fraction was measured by RIA as described in Methods.
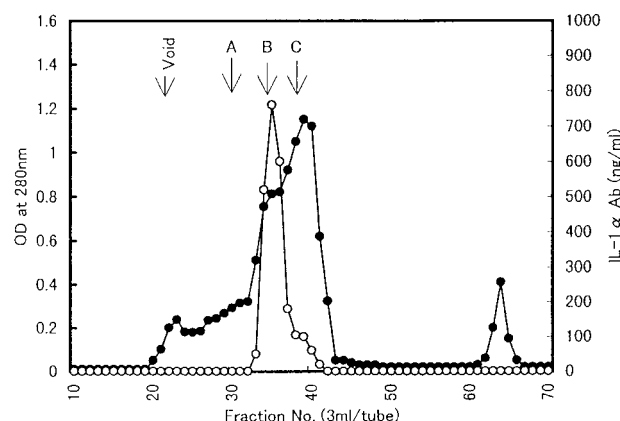

Fig. 4. Analysis of IL-1α autoantibodies by gel filtration. Plasma with high levels of IL-1α autoantibody were fractionated by gel filtration using Sepharose 6 B column. The titer of the IL-1α autoantibody in each fraction was measured by RIA as described in Methods. Closed circle indicated concentrations of protein in each fraction as determined by OD 280 ; open circle indicated concentrations of IL-1α autoantibodies in each fraction. Molecular marker : A ; Ferritin (440 Kd), B ; IgG (160 Kd), D ; albumin (68 Kd).

antibodies, whose level was over 1000 ng/ml, blocked binding of ^{125}I-labeled IL-1α to the IL-1 receptor, showing neutralizing activity against IL-1α (Fig.6). Plasma levels of IL-1α autoantibodies much less than 1000 ng/ml or without detectable titers showed no detectable neutralizing activity.

DISCUSSION

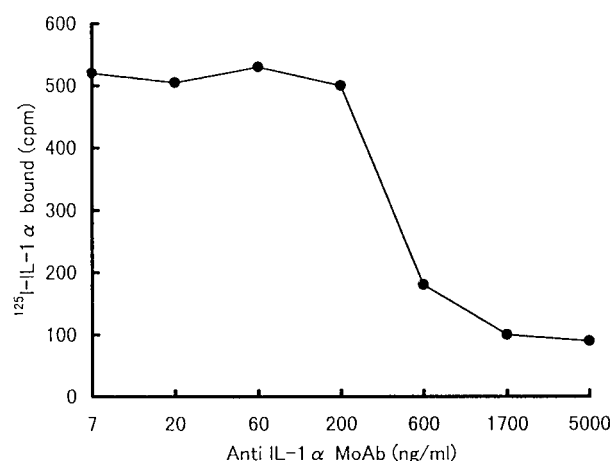High-affinity specific autoantibodies to various cytokines,

Fig. 5. A standard of IL-1α receptor assay. BALB/3T3 fibroblasts were incubated with 125 I-labeled IL-1α for 2 hr in the presence of the indicated concentrations of monoclonal antibody to IL-1α (ANOC 301), which has neutralizing activity for IL-1α. Cells were rinsed and solubilized, and then the radioactivity was counted using a γ-counter.
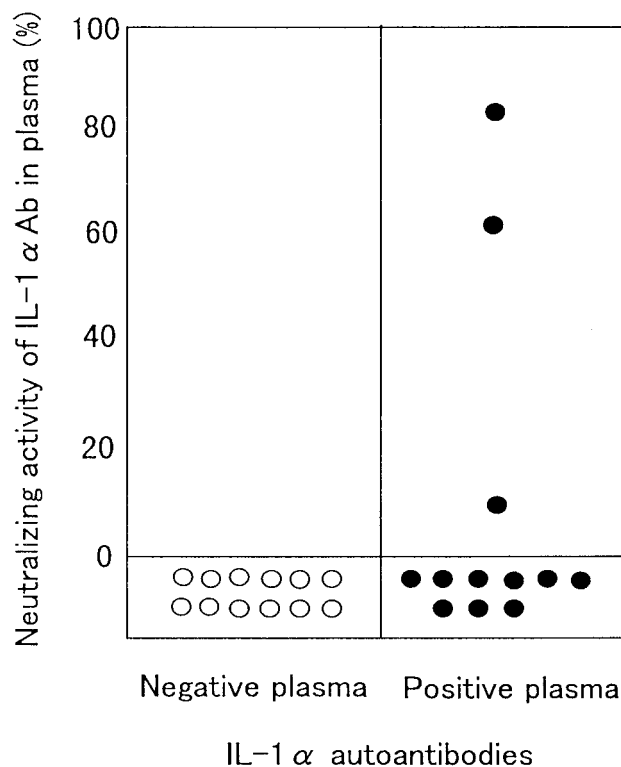

Fig. 6. Neutralizing activity of IL-1α autoantibodies in plasma. BALB/3T3 fibroblasts were incubated with ^{125}I-labeled IL-1α in the presence of plasma from normal subjects. Cells were rinsed and solubilized and then the radioactivity was counted. The subjects with a high titer of IL-1a autoantibody, whose level was over 1000 ng/ml, showed neutralizing activity.

such as IL-1α, IL-6, IL-10 and interferon, are known to be present in normal individuals (5, 6, 8-16). Recently, pharmaceutical preparations of human IgG were reported to contain antibodies against these cytokines (20, 21), indicating that blood products containing these antibodies are likely to be transfused into patients and making it necessary to clarify the prevalence of these antibodies in donor blood.

Despite several reports on the presence of IL-1α autoantibodies in normal subjects, its prevalence has been discrepant (6, 13-16). Furthermore, age-associated alterations in humoral immunity have been recognized widely, with aging associated with a decline in antibodies against pathogens and an increase in autoantibodies (22, 23). Although Hansen et al. reported that by a second-antibody precipitation assay, the prevalence of IgG antibodies against IL-1α depended on both sex and age (24), the number of subjects was apparently not enough to conclude the relation between the prevalence of IL-1α autoantibodies and age or sex. Therefore, to clarify the prevalence of IL-1α autoantibodies, we measured the level of IL-1α autoantibodies in plasma from a large number of healthy blood donors by RIA.

We found that about 15% of plasma samples from healthy donors contained detectable IL-1α autoantibodies at a level of over 5 ng/ml. Similar to other autoantibodies, their prevalence increased with age. We found their prevalence to be higher in males than in females, an unexpected difference. Therefore, the contradiction in the earlier reports concerning the prevalence of IL-1α autoantibodies may be due to the differences in the age and sex of the subjects studied. Samples with a high level of IL-1α autoantibodies (>1000 ng/ml) increased with age. However, we could not detect any IL-1β autoantibodies in plasma. With column chromatography, IL-1α autoantibodies eluted with an apparent molecular weight of 100,000-200,000 and corresponding to a subclass of the IgG fraction. Moreover, a substantial amount of this material bound to protein A which binds with high specificity to the Fc regions of IgG 1, IgG 2 and IgG 4 (25). These results suggested that IL-1α autoantibodies were of the IgG class and our results were similar to previous reports (13, 17, 26).

The role of IL-1α autoantibodies is still unknown, but reported effects include inhibition of the biologic activity of IL-1α but not IL-1β (5, 14, 17). It has been shown that Fab fragments of the antibodies bind with high affinity to human recombinant IL-1α (13, 27), and that they interfere with its T-cell stimulatory function (26). We confirmed that the plasma containing high titers of this antibodies blocked binding of ^{125}I-labeled IL-1α to IL-1 receptors on the surface of BALB/3T3 fibroblasts. Our results showed that IL-1α autoantibodies neutralize IL-1α which is consistent with other reported results (14, 16, 17). We showed that a level of IL-1α autoantibodies over 1000 ng/ml of plasma was needed to neutralize IL-1α. The affinity of the IL-1α autoantibodies in plasma examined by Scatchard analysis showed high affinity, which suggested that this antibody may play a physiological role against overproduced IL-1α .

Although the production of antibodies against IL-1α in healthy subjects can not be adequately explained, there are some interesting reports. IL-1α has been demonstrated in various body fluids and tissues, and is produced by keratinocytes and alveolar macrophages (2). IL-1α is thought to function through cell-to-cell interaction and to be involved in local immune reactions (1-3). It is suggested that IL-1α autoantibodies in some healthy subjects, produced in response to IL-1α presented in normal tissues, helps to hold the immunoinflammatory processes involving IL-1α in check. Furthermore, IL-1α autoantibodies may inhibit the systemic reaction of excess proIL-1α which has a biological function and increase in these processes. It was not clear why the prevalence of IL-1α autoantibodies in plasma was increased with age. Recently, the relation between cytokine and aging was reported (28, 29). Catania et al. showed the plasma concentration of IL-1 receptor antagonist and soluble TNF receptor were greater in healthy aged subjects than younger controls (29). We suppose that unapparent inflammation in aged subjects may cause cell activation and release of cytokine antagonist including IL-1 receptor antagonist, soluble TNF receptor and IL-1α autoantibodies.

In this study, we demonstrated the prevalence of IL-1α autoantibodies in plasma from a large number of healthy blood donors in general and by age and sex. These results would suggest that monitoring the blood donor pools for level of IL-1 α autoantibodies would be useful in disease states. Recently, cytokine inhibitor included the IL-1 receptor antagonist and the soluble IL-1 receptor was administrated to patients with immunoinflammatory diseases as a new therapeutic approach (3, 30, 31). In this regard, high concentrations of IL-1 α autoantibodies which were prepared from normal subjects may be useful as a new type of IL-1α antagonist.

ACKNOWLEDGMENTS

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