Correlation between IgG class of rheumatoid factors, immune complexes and C-reactive protein in human sera containing RF.

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Abstract

We measured the concentration of three immunoglobulin classes of rheumatoid factors (Ig-RFs), C3d binding IgG immune complex (C3d-IC), C1q binding IgG immune complex (C1q-IC) and C-reactive protein (CRP) in 74 samples of human sera with high levels of RF (24.0～2350.0 IU/ml). In sera with high levels of C3d-IC ($>15.0\mu$g/ml), there was a positive correlation between the levels of CRP and the IgG-RF, but there was no correlation between the levels of CRP and the immune complexes (C3d-IC and C1q-IC). And then, there was a positive correlation between the levels of CRP and IgG-RF or C3d-IC and IgG-RF when the levels of C1q-IC in patients sera were higher than 80.0 μg/ml. However, there was no correlation between the levels of CRP and C1q-IC in these patients sera containing high levels of both C3d-IC and C1q-IC. These results indicated that the determination of C3d-IC, C1q-IC, IgG-RF and CRP in human sera containing RF denote different implications as inflammatory indexes on progression of rheumatoid arthritis and other autoimmune diseases.

Key words : self-associating IgG rheumatoid factor, C1q binding IgG immune complex, C3d binding IgG immune complex, C-reactive protein (CRP)

INTRODUCTION

Rheumatoid factors (RF) are autoantibodies capable of binding to Fc region of denatured IgG and a little number of native IgG, and are present in the bloods and synovial fluids of the most patients with rheumatoid arthritis and other autoimmune diseases[1,2]. A number of previous studies have noted an association between immune complexes (IC) containing RF and self-associating IgG in sera and synovial fluids of rheumatoid arthritis patients and other autoimmune diseases[3-5].

Measurements of IC in sera and synovial fluids has been used as a marker for disease activity of rheumatoids arthritis. There are several methods to detect the circulating IC in sera and synovial fluids. Although, testing for circulating IC is regarded as useful laboratory parameter in the differential diagnosis and management of IC-induced disorders, there is still an uncertainty with regard to assay systems used for the demonstration of IC[6,7]. This is partly due to difficulties in the reproducibility and principle limitation of available test systems for the assessment of soluble IC in body fluids. As a result, discrepant data were

reported on the IC level of body fluids in RA dependent on the method selection.

In this study, we measured the concentration of Clq binding IgG immune complexes (Clq-IC) and C3d binding IgG immune complexes (C3d-IC) in human sera containing RF using sandwich enzyme linked immune sorbent assay (ELISA) with specific monoclonal antibodies for Clq or C3d, and tested stastically mutual relation between the rheumatoid factors (total RF, IgA-RF, IgM-RF, IgG-RF), Clq-IC, C3d-IC and C-reactive protein in each human sera.

MATERIALS and METHODS

1. Human sera as materials

The 74 samples of human sera containing rheumatoid factors (total RF with latex immune agglutination method, Kyowa Medix : 24.0 ~ 2350.0 IU/ml) are selected for this study.

2. Assay of Immune complexes in human sera

The concentrations of Clq-IC and C3d-IC were measured with ELISA method using anti-Clq monoclonal antibody or anti-C3d monoclonal antibody respectively (MBL Co. Ltd) according to the method of Antes U et al[8–10].

3. Assay of Immunoglobulin classes of RF

The values of three immunoglobulin classes of rheumatoid factors (Ig-RFs ; IgA-RF, IgM-RF and IgG-RF) in human sera were measured with ELISA method (Immunoeriot・RF for

IgM-RF and IgG-RF, IgA-RF determination reagent for IgA-RF ; Toyobo Co. Ltd).

4. CRP in human sera

The concentration of CRP was measured with the immunological turbidometric assay (Wako pure chemicals Inc).

5. Statistical analysis

The data of concentration of total RF, Ig-RFs, C3d-IC, Clq-IC and CRP in 74 patients sera were analyzed statiscally by the analysis of variance technics, and indicated the values of correlation coefficients (r) and the statiscal probability (p).

RESULTS

Distribution of data on total RF, Ig-RFs, Clq-IC, C3d-IC and CRP were shown on Table 1. And then the results of statiscal analysis of these data of r values and p values appeard on Table 2. There were slight positive correlation between the values of CRP and the C3d-IC (r= 0.505, $p < 0.001$; Fig. 1), IgA-RF and C3d-IC (r= 0.493, $p < 0.001$), IgG-RF and C3d-IC (r=0.367, $p < 0.01$) and high positive correlation between C3d-IC and Clq-IC (r=0.675, $p < 0.001$; Fig. 2) was recognized. However, there were no correlation between the values of total RF (T-RF) and the CRP, T-RF and C3d-IC or T-RF and Clq-IC. And then there were positive correlation between the levels of T-RF and the

Table 1. Mean and SD values of total RF, CRP, IgA-RF, IgM-RF, IgG-RF, Clq-IC and C3d-IC in all patients sera.

	T-RF IU/ml (n=74)	CRP μg/ml (n=74)	IgA-RF IU/ml (n=74)	IgM-RF IU/ml (n=74)	IgG-RF IU/ml (n=74)	Clq-IC μg/ml (n=74)	C3d-IC μg/ml (n=50)
mean $\bar{M}$	151.87	11.27	22.67	159.49	62.55	35.59	0.92
$\bar{M}$ ± SD	66.35 ~ 347.58	2.03 ~ 62.68	4.56 ~ 112.62	49.85 ~ 510.30	18.54 ~ 573.64	12.48 ~ 101.49	0.03 ~ 34.09

Table 2. The values of coefficients correlation (r) and the significant probability (p) in all patients sera.

|		T-RF	CRP	Clq-IC	C3d-IC	IgA-RF	IgM-RF	IgG-RF
T RF	r		0.090	0.067	0.207	0.608	0.565	0.612
	p		N. S	N. S	N. S	<0.001	<0.001	<0.001
C R P	r	0.090		0.139	0.505	0.404	0.373	0.293
	p	N. S		N. S	<0.001	<0.001	<0.001	<0.01
Cl q IC	r	0.067	0.139		0.675	0.184	0.043	0.186
	p	N. S	N. S		<0.001	N. S	N. S	N. S
C3 d IC	r	0.207	0.505	0.675		0.493	0.278	0.367
	p	N. S	<0.001	<0.001		<0.001	<0.05	<0.01
Ig A RF	r	0.608	0.404	0.184	0.493		0.713	0.644
	p	<0.001	<0.001	N. S	<0.001		<0.001	<0.001
Ig M RF	r	0.565	0.373	0.043	0.278	0.713		0.662
	p	<0.001	<0.001	N. S	N. S	<0.001		<0.001
Ig G RF	r	0.612	0.293	0.186	0.367	0.644	0.662	
	p	<0.001	<0.01	N. S	<0.01	<0.001	<0.001	
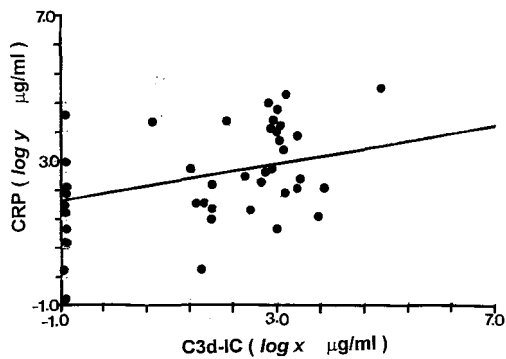
Fig. 1. Significant correlation between the concentration of C3d-IC and the CRP in patients sera. $Y = 0.26X + 2.17$, $r = 0.505$, $p < 0.001$, $n = 50$.
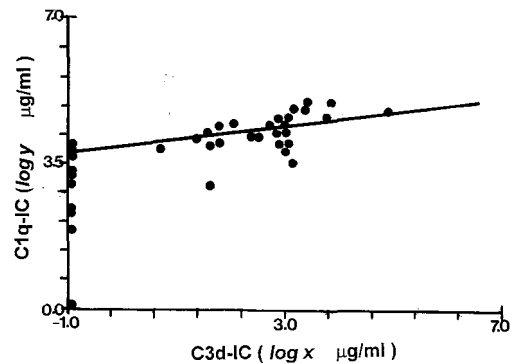

Fig. 2. Significant correlation between the concentartion of C3d-IC and the Clq-IC in patients sera. $Y = 0.16X + 3.94$, $r = 0.675$, $p < 0.001$, $n = 50$.

Ig-RFs　(T-RF : IgA-RF,　r=0.608,　p<0.001. T-RF : IgM-RF, r=0.565, p<0.001. T-RF : IgG-RF, r=0.612, p<0.001).

When the concentration of C3d-IC was higher than 15.0μg/ml (n=20), there was positive correlation between CRP and IgG-RF (r=0.630, p<0.001 ; Fig. 3). And also, when the concentration of C1q-IC was higher than 80.0 μg/ml (n=16), there were positive correlation between the levels of CRP and the IgG-RF (r = 0.606, p <
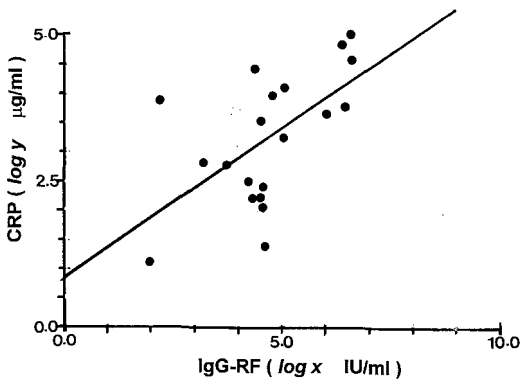

Fig. 3. Significant correlation between the concentration of CRP and the IgG-RF in patients sera containing high levels of C3d-IC. (C3d-IC ; >15. 0μg/ml, n=20)
Y=0.51X+0.81, r=0.598, p<0.005.

Fig. 4. Significant correlation between the concentration of CRP and the IgG-RF in patients sera containing high levels of C1q-IC. (C1q-IC ; >80. 0μg/ml, n=16)
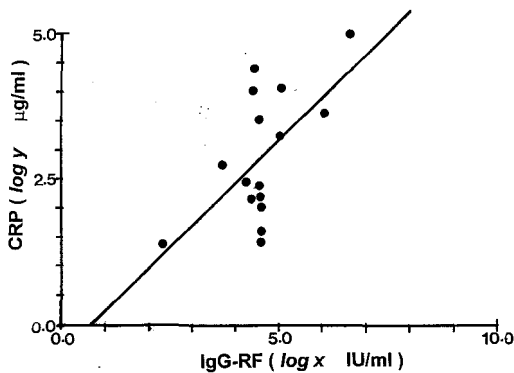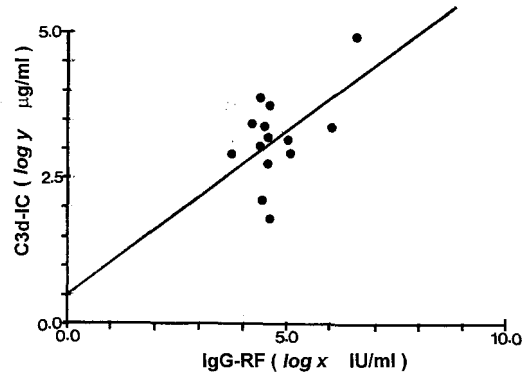Y=0.74X−0.47, r=0.607, P<0.01.

Fig. 5. Significant correlation between the concentration of C3d-IC and the IgG-RF in patients sera containing high levels of C1q-IC. (C1q-IC ; >80. 0μg/ml, n=16)
Y=0.55X+0.52, r=0.655, P<0.005.

0.01 ; Fig.4), and between C3d-IC and IgG-RF (r=0.655, p<0.005 ; Fig.5). However, there was no correlation between the levels of CRP and the C1q-IC in these patients sera containing high levels of both C3d-IC and the C1q-IC.

DISCUSSION

The pathogenic mechanisms in rheumatoid arthritis (RA) include humoral and cellular immunity. The humoral immunity participates in the pathogenesis of RA through formation of immune complexes. In patients with RA, immune complexes have been demonstrated in circulation, synovial fluids, synovial tissue, phagocytic cells in the synovial lining and superficial layers of articular cartilage. RFs are abundantly formed in the antigen-antibody complexes at these locations, indicating their participation in the inflammatory lesions of RA.

IgG-RF was initially recognized as constituents of the intermediate complexes in the serum of patients with RA. IgG-RF forms immune complexes by self-association, a unique process that allows immune complex formation without

the presence of antigen molecules. The interaction of two IgG-RF molecules is stabilized by the formation of two antigen-antibody bonds between them, leaving free antibody combining sites and free antigenic determinants.

Interaction of IgM-RF with monomeric or polymeric IgG may lead to formation of immune complexes of varying sizes in sera and synovial fluids of patients with RA. The nature of the immune complexes formed between IgA-RF and monomeric IgG was not examined. Thus, IgM-RF and IgG-RF in immune complexes can contribute to inflammation by activating the complement system.

The appearance of CRP in the serum is a nonspecfic response to inflammation, infection, and tissue damage. CRP which is one of the "acute phase protein" which of the concentration are rised in acute phase sera and synovial fluids of patients with RA. CRP can also bind to a variety of ligands of autogenous orgin including phospholipids, nonphospholipids, polyanion and polycations. A major function of CRP may be to recognize such autogenous materials in the plasma following tissue damage and to detoxify them for their rapid clearance. Because of the ability of aggregated or complexed human CRP to activate the classical complement pathway, it was also raised as a possibility that CRP could enhance inflammation and tissue damage. Complexed human CRP activated the first component of human complement to bind the N-terminal of C1q molecules[11].

Our previous studies demonstrated the high correlation between the levels of total RF (measured with RAPA) and Ig-RFs (ELISA) in human sera $(r=0.90)$[12,13]. In this study, we confirmed high correlation between the levels of total RF (Latex Immuno turbidometric assay) and the IgG-RF $(r=0.612)$. There was

some positive correlation between the levels of IgG-RF and the C3d-IC (data not shown), but not between the IgG-RF and C1q-IC for 74 samples. However there was no correlation between the levels of C3d-IC and the IgG-RF in patients sera containing high levels of C3d-IC, on the contrary, there was positive correlation between the levels of C3d-IC and the IgG-RF in the patients sera containing high levels of CIq-IC. In addition, there was positive correlation between the levels of C3d-IC and the CRP for 74 samples, but no correlation between the C3d-IC and the CRP in the patients sera containing high levels of C3d-IC and C1q-IC (data not shown). These results indicated that the determination of C3d-IC, C1q-IC, IgG-RF and CRP in human sera shows different implications as inflammatory indexes on progression of rheumatoid arthritis[14-16]. The results indicate if when we detect the high levels of C3d-IC and the C1q-IC in patients sera, caution should be exercised to evaluate the concentration of CRP in such cases. The above date are conformed well to the study of Watanabe et al[17], that support the usefulness of C3d-IC and IgG-RF measurements for the prediction of progressive disorder in RA. These observations suggest the necessity of measuring the levels of C3d-IC, C1q-IC, IgG-RF and CRP at the same time in patients sera and synovial fluids of RA.

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リウマチ因子陽性ヒト血清中の免疫グロブリンＧクラスリウマチ因子と免疫複合体ならびに CRP との相関性

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要　　約

　一般に慢性関節リウマチの診断ならびに活動性の一指標として，日常臨床検査では患者血清中のリウマチ因子の測定が実施されている。

　本論文では RF 検査依頼が有り，高 RF 値（24.0〜2350.0 IU/ml）を示した患者血清74検体について，免疫グロブリンクラス別 RF 値を測定するとともに自己 IgG と補体との免疫複合体 (IC) である C1q-IgG・IC (C1q-IC)，C3d-IgG・IC (C3d-IC) ならびに CRP 値を測定し，それらの測定値間の相関性

について統計学的検討を行った。C3d-IC 値が15.0μg/ml 以上の高値を示す患者血清では，CRP 値と IgG クラスの RF(IgG-RF)値間については正の相関結果が得られた。また，C1q-IC 値が80.0μg/ml 以上の場合にも CRP 値と IgG-RF 値間および C3d-IC 値と IgG-RF 値間では正の相関結果が得られた。しかし，C3d-IC ならびに C1q-IC ともに高値例の患者血清中の CRP 値と C1q-IC 値間には有意な相関は観察されなかった。以上の結果より，慢性関節リウマチの活動度を判定する上で，従来から炎症マーカーとして CRP 値が利用されているが，血清中に免疫複合体が高レベルに検出される患者については，炎症の指標として CRP 以外にも C1q-IC や C3d-IC および IgG-RF 等を加えた総合的な判断が必要であることが示唆される。

キーワード：自己 IgG 結合性リウマチ因子，C1q 結合 IgG 免疫複合体，C3d 結合 IgG 免疫複合体，
　　　　　　　C 反応性タンパク質（CRP）

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