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SHORT COMMUNICATION

Elevation of serum IgG subclass concentration in patients with rheumatoid arthritis

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Abstract A new concept of IgG subclass such as IgG4-related systemic disease including autoimmune pancreatitis, characterized by a high-serum IgG4 level, has been proposed. Our aim was to investigate serum IgG subclass levels in rheumatoid arthritis (RA). IgG subclass levels of sera from 72 patients with rheumatoid arthritis were determined by immunonephelometric assay. The patients were divided into two groups according to clinical activity of the disease: active disease and remission. The sera from 45 normal controls were also measured. All statistical analyses were carried out with SPSS software version 13.0. We found a significant increase of all the four IgG subclass concentrations in sera of patients with RA compared to those of the controls ($P < 0.001$). When the patients were divided according to clinical activity, the IgG subclass concentrations were similar between the two groups ($P > 0.05$). Our data suggest that besides IgG1, IgG2 and IgG3, IgG4 may involve in the pathogenesis of the disease.

Keywords Rheumatoid arthritis · IgG subclass · Immunonephelometric assay

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by the progressive and irreversible destruction of joints. The pathogenesis of rheumatoid arthritis is still an unsolved puzzle. Elevation of serum IgG4 concentration is well known in autoimmune pancreatitis (AIP) [1]. In AIP, increased numbers of IgG4 positive plasma cells are associated with the pathology of diseases. In patients with RA, subclass distribution of the IgG response to different antigens has been reported [2]. Therefore, examination of the patterns of distribution of serum IgG subclass concentrations may provide insight into the immunological process involved in RA. Two reports have described the IgG subclass concentrations profiles in sera from patients with RA [3, 4]. Results from these studies were discrepant. In the past, IgG subclass measurements were generally performed with radial immunodiffusion (RID). With this method, results are obtained after an incubation period of 48–72 h. In this study, we measured the serum levels of IgG subclasses in patients with RA by immunonephelometric assay, which allows a quantification of IgG subclass concentrations in a large number of samples quickly (less than 15 min for a complete IgG subclass profile), reproducibly and precisely [5].

Materials and methods

Patients

Seventy-two patients, 12 males and 60 females, ranging in age from 27 to 64 years were enrolled in this study. The patients fulfilled the American College of Rheumatology (ACR) classification criteria for RA [6]. Remission was

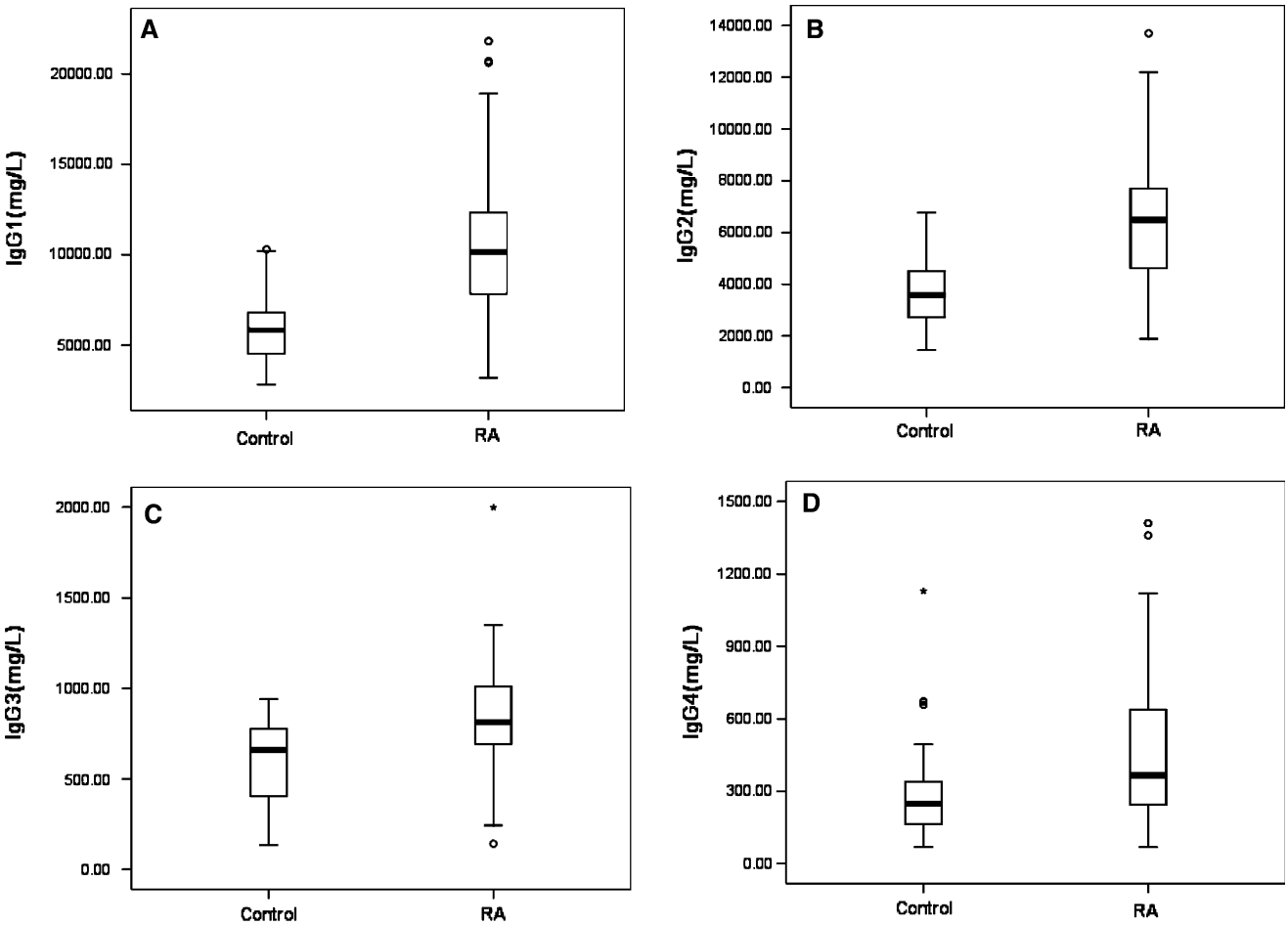

Fig. 1 Serum IgG concentrations of 45 controls and 72 RA patients

defined according to the Rheumatoid arthritis Disease Activity Index (RADAI) [7]. We categorized RA patients as the "active group" ($n = 54$) and "remission group" ($n = 18$). The results were compared with values obtained from 45 normal controls (10 males and 35 females, age 20–60). The study was approved by the Local Ethics Committee and written informed consent was obtained from all patients prior to serum sampling.

Measurements of serum IgG subclass concentrations

Serum IgG subclass levels were determined by immunonephelometric assay with a kit (The Binding Site Ltd, Birmingham, UK) and the Immage®800 (Beckman Coulter, Brea, CA, USA) Analyser.

Statistical analysis

Data were expressed as median (5th and 95th percentiles). Differences in variables between different groups were compared statistically by the Mann–Whitney non-parametric *U* test. Probability values of less than 0.05 were considered statistically significant. All statistical analyses were carried out with SPSS software version 13.0.

Results

Results of IgG subclass distribution in RA patients are illustrated graphically in Fig. 1 in comparison with normal controls. Significantly higher values of IgG1, IgG2, IgG3 and IgG4 were observed ($P < 0.001$) (Table 1).

When patients were divided into two groups according to clinical activity of the disease (active disease and remission), the serum IgG1, IgG2, IgG3 and IgG4 concentrations were similar between the two groups ($P > 0.05$) (Table 2).

Discussion

In the present study, we evaluated IgG subclass levels in sera from 72 patients with RA. Serum IgG1, IgG2, IgG3

Table 1 Serum IgG concentrations of normal controls and patients with RA

	Normal controls ($n = 45$)	Patients with RA ($n = 72$)	P* value
Median (5th, 95th percentiles)			
Serum IgG1–mg/L	5,840 (3,439, 10,056)	10,150 (4,769, 21,085)	<0.001
Serum IgG2–mg/L	3,590 (1,575, 6,167)	6,495 (2 368, 11,640)	<0.001
Serum IgG3–mg/L	658 (204, 916.6)	814.5 (344.8, 1,264)	<0.001
Serum IgG4–mg/L	248 (68.1, 672.1)	365.5 (72.85, 1,377.5)	<0.001

* The Mann–Whitney non-parametric U test was used to calculate two-sided P values

Table 2 Serum IgG concentrations of patients in remission and active disease

	Active disease ($n = 54$)	Remission ($n = 18$)	P* value
Median (5th, 95th percentiles)			
Serum IgG1–mg/L	10,600 (4,750, 21,800)	8,830 (4,840, 13,000)	0.138
Serum IgG2–mg/L	6,390 (2,322.5, 11,675)	6,535 (2,290, 11,900)	0.765
Serum IgG3–mg/L	805.5 (284.25, 1 305)	841 (383, 1,140)	0.730
Serum IgG4–mg/L	391.5 (73.58, 1 410)	355 (68.1, 921)	0.572

* The Mann–Whitney non-parametric U test was used to calculate two-sided P values

and IgG4 were significantly higher in RA than in healthy people. All of these three IgG subclasses ($P < 0.001$) presented the striking increase. The finding suggests a major role for these IgG subclasses in the inflammatory process of RA.

Our results are different from those of previous studies. One group reported low levels of IgG2 and IgG4, and raised levels of IgG1 with normal levels of IgG3 in adult RA [4]. However, the group of Martini found raised levels of IgG1, IgG2 and IgG3 with normal levels of IgG4 in juvenile chronic arthritis [3, 8]. The discrepancies may be explained by different assay protocol used (Martini used radial immunodiffusion) or differences in the selection of the patient populations for study.

The four IgG subclasses have distinct biological properties [9]. IgG1 and IgG3 are able to activate all types of Fc receptors and the C1 component of complement (C1q). IgG2 has restricted Fc receptor and C1 activating abilities. IgG4 has restricted Fc receptor and does not activate C1q. It might be expected that IgG1 and IgG3 would be mainly involved in the immunopathology associated with IgG-mediated autoimmune inflammatory conditions. IgG4 seems to be the most inert among the IgG subclasses. However, we found a significant elevation of serum IgG4 concentration in patients with RA compared to those of normal controls. We can infer from the results that IgG4 is involved in the immunopathology of RA. It has been demonstrated that IgG4 significantly outweighs IgG2 and IgG3 among anti-cyclic citrullinated peptides (CCP) antibodies in RA patients [10]. Rheumatoid factor (RF) is often evaluated in patients suspected of RA. IgG1 and IgG4 were found to be the predominant IgG subclasses against RF [11]. So far, an active pathological role for IgG4 has only been described for blistering skin disease [12]. However, IgG4 autoantibody has the potential pathological function in the autoimmune process. IgG4 was proved to be able to bind to human IgG1, IgG2, IgG3 and IgG Fc through its own Fc [13]. IgG4 Fc–Fc binding may involve in the inflammatory process by inducing aggregation of immunoglobulins. Another possibility is that IgG4 could block Fc-mediated effector functions of IgG1 and IgG3, and may dampen the inflammatory response. Furthermore, IgG4 molecule has shown to be able to change Fab arms in vivo [14]. The main effect of this Fab-arm exchange is the generation of monovalent, non-cross-linking antibodies. In a mouse model of myasthenia gravis, IgG4 was protective due to its acquired monovalency by competition with a pathogenic IgG1 antibody [14]. Studies showed that it usually takes many months of repeated antigen exposure before IgG4 responses become prominent [15]. In RA, the prolong immunization of autoantibodies may be potential for IgG4 antibody production. In conclusion, significant increase of IgG4 in RA did prove that IgG4 involves in the immune process of the disease. In this process, IgG4 may be just the product of the prolong immunization or may play roles as protective antibodies.

When our patients were divided according to clinical activity of the disease, no significant difference was observed between the patients in active disease and patients in remission, a phase that we might expect to be associated with progressive reduction of the inflammatory process. It is hard to explain the results since no decrease of IgG subclass concentration was observed.

In conclusion, determination of IgG subclass concentrations in RA seems to provide a useful insight into the pathological process operating in the disease. In addition, the pathological function of IgG4 autoantibodies in RA needs to be clarified.

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