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IgA rheumatoid factor in mucosal fluids and serum of patients with rheumatoid arthritis: immunological aspects and clinical significance

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SUMMARY

In order to gain insight into the production and clinical significance of IgA rheumatoid factor (IgA-RF) in mucosal fluids of patients with rheumatoid arthritis (RA), we examined tear fluid, saliva and serum from 80 patients with RA. Significant correlations were found between IgA-RF levels in tear fluid and saliva ($P=0{\cdot}002$, $r=0{\cdot}57$), saliva and serum ($P<0{\cdot}001$, $r=0{\cdot}79$), and serum and tear fluid ($P<0{\cdot}001$, $r=0{\cdot}31$). No significant correlations were found between total IgA levels in these fluids. Comparison between circulating and mucosal IgA-RF levels after correction for total IgA, revealed that mucosal IgA-RF levels are on average 2·5 times higher than circulating IgA-RF levels. Analysis of IgA-RF specificity showed that lacrimal and salivary IgA-RF reactivity with various IgG subclasses is similar and differs from serum IgA-RF specificity. These results indicate local production of IgA-RF in salivary and lacrimal glands and support the view of a common origin of IgA-RF producing B cells present in mucosal tissues. Mucosal and circulating levels of IgA and IgA-RF were not associated with tests that quantify tear fluid production. This indicates that mucosal and circulating levels of IgA and IgA-RF in patients with RA cannot be regarded as markers for the development of secondary Sjögren's syndrome.

Keywords IgA rheumatoid factor mucosal fluids rheumatoid arthritis

INTRODUCTION

Increased levels of IgA rheumatoid factors (IgA-RF) can frequently be found in serum from patients with rheumatoid arthritis (RA) [1,2]. IgA-RF has also been demonstrated in mucosal fluids of patients with RA [2]. Measurement of IgA-RF in RA may prove to be clinically relevant because of the reported association with disease manifestations. In serum, increased IgA-RF levels were found to be associated with progressive joint destruction and in mucosal fluids IgA-RF levels of patients with RA complicated with Sjögren's syndrome or sicca syndrome (the ocular part of Sjögren's syndrome) were reported to be higher than in mucosal fluids of patients with uncomplicated RA [3–7]. It is not known whether in patients with RA the production of IgA-RF at mucosal sites is the result of a generalized immune reponse or of a local stimulation of the mucosal immune system.

The aim of this study was to obtain information on the regulation of mucosal IgA-RF production. IgA-RF was studied by comparing levels of IgA and IgA-RF as well as the reactivity of IgA-RF with IgG subclasses between samples of tear fluid, saliva and serum of individual patients. In addition, the clinical significance of IgA-RF measurement in RA was studied by comparing IgA-RF levels in mucosal fluids of RA patients with the presence of signs and symptoms of sicca syndrome.

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PATIENTS AND METHODS

Patients

Paired samples of serum, saliva and tear fluid were obtained from 80 patients with classic or definite RA as defined by the criteria of the American Rheumatism Association [8]. Forty-two of the RA patients were selected because of complaints of dry eyes and/or a dry mouth. Using the Schirmer's test 42 of the 80 patients were found to have an abnormal tear production defined as less than 5 mm wetted paper in 5 min. In 38 cases an abnormal tear film break up time (BUT) was diagnosed, defined as a break in the tear film within 10 s. Sixteen patients who complained about dry eyes were found to have abnormal test results in both the Schirmer's and BUT test. In addition, samples of serum, saliva and tear fluid from 15 control patients (five patients with ankylosing spondylitis and 10 patients with osteoarthritis) were also studied.

Known volumes of tear fluid were collected and diluted with buffer to a total volume of 100 μl; patients were excluded when no more than 5 μl of tear fluid could be collected. Diluted tear

fluid and samples of stimulated saliva were centrifuged at 100 *g* for 5 min at room temperature. Serum samples and supernatant from both saliva and diluted tear fluid were stored at −20°C until the assays were performed.

Reagents

Horseradish peroxidase (HRP)-conjugated goat IgG anti-human IgA (goat anti-human IgA HRP), goat anti-human IgG HRP and four different human myeloma IgG preparations representing all four human IgG subclasses were purchased from Nordic (Tilburg, The Netherlands). Buffers, MoAbs and other reagents were prepared or obtained as described previously [9].

ELISA for measurement of IgA-RF

Flat-well microtitre plates were coated with 10 μg/ml human IgG and then incubated with diluted test samples for 2 h at 37°C. The plates were then incubated with biotin-conjugated MoAb directed against human IgA (clone 4E8) [9] for 1 h at 37°C and thereafter with streptavidin-HRP for 1 h at 37°C. Subsequently, the plates were incubated with tyramine conjugated with biotin for 1 h at 37°C followed by streptavidin-HRP for 1 h at 37°C. The colour reaction using OPD and conversion of optical densities into arbitrary U/ml of IgA-RF was performed as described previously [9].

To ensure that results of IgA-RF measurement were not due to aspecific binding of IgA, samples of serum, saliva and tear fluid from 10 patients with RA with high IgA-RF levels were tested by ELISA. Test plates were coated with buffer, rabbit IgG, human IgG-Fc, human IgG Fab, mouse IgG or albumin, and processed as described above. All samples gave a positive reaction in ELISA on wells coated with human IgG-Fc and rabbit IgG, but not on wells coated with human IgG-Fab, mouse IgG, albumin and buffer. These results indicate that results of IgA-RF measurements were not due to aspecific binding of IgA (data not shown).

ELISA for measurement of IgG subclass specificity of IgA-RF

To measure IgA-RF reactivity with different IgG subclasses, ELISA wells were coated with human IgG1, IgG2, IgG3 and IgG4. To ensure that on the ELISA test plates all four human IgG subclasses were coated to the same extent, coated plates were incubated with either an HRP-labelled mouse MoAb directed against all four human IgG subclasses, goat anti-human IgG HRP, rabbit anti-human IgG HRP or protein A HRP (which only binds to human IgG1, IgG2 and IgG4). These different conjugates were employed as an internal control to avoid intrinsic bias of IgG subclass specificity. IgG1 coated at a concentration of 1·25 μg/ml, IgG2 at 1·25 μg/ml, IgG3 at 1·25 μg/ml and IgG4 at 2·5 μg/ml yielded identical ODs after incubation with an individual conjugate. Subsequently IgA-RF levels were measured in paired samples of saliva, tear fluid and serum derived from 21 patients with RA of whom enough tear fluid was left to examine. To demonstrate that binding of IgA-RF to IgG subclasses was not due to aspecific binding of IgA, samples of serum, saliva and tear fluid from 10 patients with RA and 15 control patients were tested on ELISA test plates coated with human IgG subclasses. Samples derived from patients with RA gave OD values clearly different from background values, whereas samples from control patients mostly yielded OD values close to zero (data not shown).

Results of IgG subclass reactivity of IgA-RF were expressed as follows. After subtraction of background values, ODs of IgA-RF reactivity of a sample from one patient on four IgG subclasses were added up, and set on 100% for this particular sample. Using this 100% value, IgA-RF subclass reactivity on all four IgG subclasses was converted from ODs into percentage reactivity. This was done for each individual sample tested for IgG subclass reactivity. Samples yielding an OD below 0·1 when tested on an IgG subclass were considered as not reactive with that particular subclass. By this method, not only the reproducibility of the assay improves, but a comparison of subclass reactivity between different patient samples can be made without absolute differences in IgA-RF levels obscuring values of IgG subclass reactivity as would occur when IgG subclass reactivity values were expressed in ODs.

ELISA for determination of IgA concentrations

Microtitre plates were coated with 10 μg/ml of 4E8 and subsequently incubated with diluted test samples for 1 h at 37°C. Dilutions of a reference serum (Central Laboratory Bloodtransfusion, Amsterdam, The Netherlands) were run with each assay to construct an IgA standard curve. Subsequently, the plates were incubated with goat anti-human IgA-HRP. The colour reaction and detection were performed in the same manner as described above.

Optical densities of the test serum, saliva or tear fluid were taken from only the steep portion of the standard curve and were converted into U/ml IgA-RF or mg/ml IgA.

Comparison of RF levels in tear fluid, saliva and serum

To compare the levels of IgA-RF in saliva or tear fluid with those in serum, we corrected for local IgA synthesis and/or passive flow between the compartments using IgA concentrations found in the three body fluids. The relative concentration ratio (RCR) of IgA-RF compared with IgA was calculated according to the following formulae:

$$\text{Saliva:serum} = \frac{\text{IgA-RF in saliva}}{\text{IgA-RF in serum}} \times \frac{\text{IgA in serum}}{\text{IgA in saliva}}$$

and

$$\text{Tear fluid:serum} = \frac{\text{IgA-RF in tear fluid}}{\text{IgA-RF in serum}} \times \frac{\text{IgA in serum}}{\text{IgA in tear fluid}}$$

Statistical analysis

Spearman's rank correlation was used to compare the levels of immunoglobulins in serum, saliva and tear fluid. The Mann–Whitney *U*-test was used to compare levels of immunoglobulins between RA patients with different disease classification.

RESULTS

Concentrations of IgA and IgA-RF as determined in paired samples of serum, saliva and tear fluid from 80 patients with RA are shown in Fig. 1a and b. Sixty-nine percent of the sera tested contained elevated levels of IgA-RF. Comparison of immunoglobulin levels in different body fluids from RA patients revealed significant correlations between IgA-RF levels in saliva and tear fluid ($P = 0{\cdot}002$, $r = 0{\cdot}57$), serum and saliva ($P < 0{\cdot}001$, $r = 0{\cdot}79$) and serum and tear fluid ($P < 0{\cdot}001$, $r = 0{\cdot}31$). No significant correlations were found for levels of total IgA between the three body fluids. IgA-RF levels were also compared between serum, saliva and tear fluid after correction for local IgA synthesis using IgA concentrations present in each

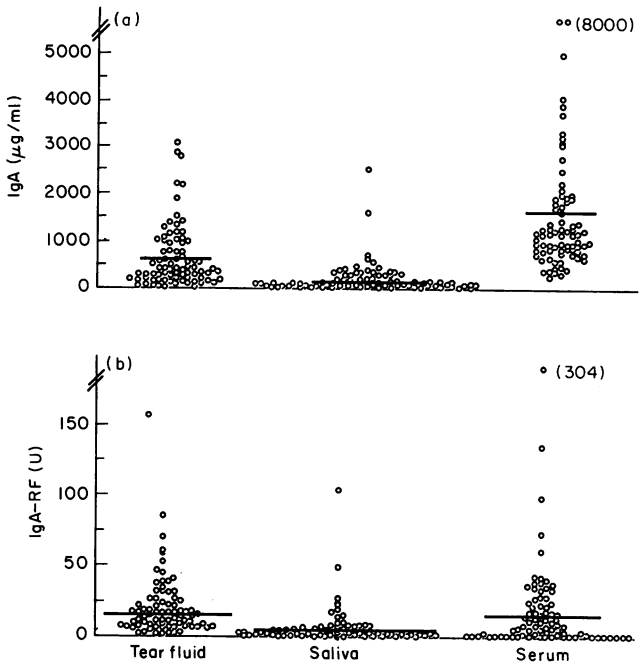

Fig. 1. Paired samples of tear fluid, saliva and serum derived from 80 patients with rheumatoid arthritis (RA) were assayed for the presence of total IgA (a) and IgA-RF (b). Each point represents the value of IgA or IgA-RF in one sample. The mean IgA or IgA-RF values are depicted as a straight line.

body fluid. For IgA-RF the saliva:serum RCR was 2·7 and the tear fluid:serum RCR was 2·5.

To examine the specificity of IgA-RF present in mucosal secretions and in the circulation, we compared IgA-RF anti-IgG subclass reactivity between paired samples of tear fluid, saliva and serum from 21 patients with RA (Fig. 2). This comparison demonstrated that lacrimal and salivary IgA-RF reacts significantly better with IgG1 and IgG4 than with IgG2 and IgG3 (Fig. 2a, b). In contrast, IgA-RF present in sera from the same patients was found to react significantly better with IgG2 than with IgG1 and IgG4, whereas the reactivity of circulating IgA-RF with IgG3 varied considerably between individual patient samples (Fig. 2c).

To assess the clinical significance of IgA-RF in tear fluid, saliva and serum of patients with RA, we examined whether RA patients who scored positive for parameters indicative for sicca syndrome could be identified from RA patients scoring negative for these parameters by measuring IgA-RF and IgA levels in these body fluids. When levels of IgA-RF and IgA in the different body fluids were compared between patients with or without complaints of dry eyes or mouth, positive or negative Schirmer's test and positive or negative BUT-test, no significant differences were found. Comparison of IgA and IgA-RF levels present in the three body fluids between RA patients with and without any combination of sicca complaints and abnormal results of ophthalmologic test revealed no significant differences. Conversely, no significant differences were found for the presence of any clinical parameter measured between RA patients with high and low levels of serum, salivary or lacrimal IgA or IgA-RF.

DISCUSSION

Studies of IgA-RF in RA have thus far mainly concentrated on circulating IgA-RF [1–5,9]. Although IgA-RF has been shown to occur also in mucosal fluids from patients with RA, little is known about the origin, regulation of production and clinical relevance of mucosal IgA-RF.

In humans, the bone marrow and spleen are the major sources of serum IgA whereas IgA in external secretions is produced by cells distributed in the tissues of secretory glands [10]. The production of specific antibodies secreted in mucosal fluids is either the result of a local antigenic stimulus or of antigenic stimulation of the Peyer's patches, a specialized organ in the small intestine regulating the mucosal immune responses [11]. A local antigenic stimulus results in local production of antigen-specific IgA antibodies. Antigen presentation in the Peyer's patches may result in priming of antigen-specific B cells which subsequently home via the lymph nodes and circulation to distant mucosal tissues [12]. This mechanism, which leads to the presence of antibodies with identical specificity at different mucosal sites, is often referred to as the common mucosal immune system [13]. As a consequence, antigen-specific IgA antibodies can be found in mucosal fluids and not in the circulation, or *vice versa* [10]. However, the regulation of production of mucosal and circulating IgA antibodies is not considered to be fully independent. Continuous oral administration of antigen induces not only the production of mucosal antigen-specific IgA, but also of circulating antigen-specific IgA. In addition, oral administration of an antigen previously encountered in the circulation also yields circulating antigen-specific IgA [13]. By screening serum and two different mucosal fluids for specific antibody activity, insight may be gained into the regulation of production of a particular antibody.

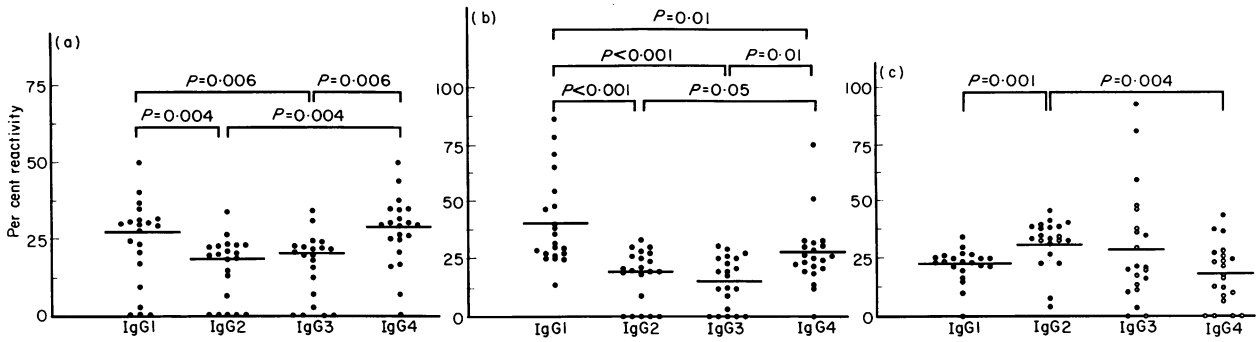

Fig. 2. Reactivity of IgA-RF present in paired samples of tear fluid (a), saliva (b) and serum (c) derived from 21 rheumatoid arthritis (RA) patients with human IgG1, IgG2, IgG3 and IgG4. The mean values are indicated with a straight line.

By measuring IgA and IgA-RF levels in tear fluid, saliva and serum of 80 patients with RA, significant correlations were found between levels of IgA-RF present in saliva, tear fluid and serum, but not between levels of total IgA in the three body fluids. This suggests that the correlations between levels of IgA-RF in the different body fluids are not due to passive IgA exchange. Combined with the fact that the RCR values of IgA-RF compared with IgA in saliva and tear fluid are more than 1·0, the data indicate a local production of IgA-RF in the salivary and lacrimal glands as well as a relation between the production of IgA-RF in both exocrine glands. The data support the view that IgA-RF-producing B cells present in mucosal tissues share a common origin. The finding that IgA-RF present in both tear fluid and saliva differs from serum IgA-RF regarding the extent of reactivity of IgA-RF with human IgG subclasses, might be interpreted as additional suggestive data for the involvement of the common mucosal immune system in the production of mucosal IgA-RF.

The significant relation between circulating and salivary/lacrimal IgA-RF levels also suggests a relation between the mucosal immune system and the production of circulating IgA-RF. Such a relation has already been suggested in previous studies on IgA-RF. First, IgA-RF present in serum and saliva from patients with RA mainly occurs in the polymeric form whereas 10% of circulating IgA and 90% of IgA in mucosal fluids is polymeric [1,2]. Second, polymeric IgA-RF present in serum of 129/SV mice was shown to originate from lymph nodes draining the intestinal tract [14]. Other studies, however, by demonstrating that circulating IgA-RF mainly consists of the IgA1 subclass [2,15] suggested that IgA-RF originates from the bone marrow or from inflamed synovium. Both compartments produce IgA mainly of the IgA1 subclass, whereas in mucosal fluids approximately equal amounts of IgA1 and IgA2 can be found [10]. The production site of serum IgA-RF has not yet been studied in detail. Further studies on IgA-RF production by circulating and bone marrow plasma cells should provide more detailed information on the source of serum IgA-RF in patients with RA.

In previous studies it was reported that in mucosal fluids from RA patients complicated with Sjögren's syndrome or sicca syndrome significant higher levels of IgA-RF can be found than in mucosal fluids from patients with uncomplicated RA [6,7]. Given the fact that up to one-third of RA patients may develop sicca syndrome, it was suggested that mucosal IgA-RF could be employed as a marker for this complication [6]. In the present study, we could not distinguish RA patients with signs and symptoms of the sicca syndrome from RA patients without the sicca syndrome by means of mucosal or circulating IgA and IgA-RF levels. In this study salivary gland biopsies were not performed to obtain histological evidence for the presence of Sjögren's syndrome. However, the number of patients studied with signs or symptoms of sicca syndrome was relatively large, and the finding that even patients with both complaints of dry eyes and two abnormal tests for tear fluid production did not have increased IgA-RF or IgA levels in tear fluid compared with patients without these parameters indicates that increased production of IgA-RF or IgA at mucosal sites is not related to the exocrine gland inflammation in a sicca syndrome. On the contrary, the results seem to suggest a decreased mucosal production of both IgA-RF and IgA in patients with sicca syndrome. An unaffected production of IgA-RF and IgA in the presence of a decrease in salivary and lacrimal flow rate would result in an elevation of salivary and lacrimal IgA-RF and IgA levels, which was not the case. This implies that the inflammatory disorder which lies at the basis of the sicca syndrome also affects production of mucosal IgA-RF and IgA.

The data presented indicate local production of IgA-RF in salivary and lacrimal glands of patients with RA, and suggest involvement of the common mucosal immune system in the production of mucosal IgA-RF. The fact that no relation was found between mucosal IgA-RF or IgA levels and signs and symptoms of sicca syndrome indicates that measurement of mucosal IgA and IgA-RF cannot be employed as an objective parameter in the diagnosis of sicca syndrome.

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