Immunoglobulin G and A Antibody Responses to *Bacteroides forsythus* and *Prevotella intermedia* in Sera and Synovial Fluids of Arthritis Patients

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The objective of the present study was to investigate immunoglobulin G (IgG) and IgA antibody immune responses to *Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus,* and *Candida albicans* in the sera of patients with rheumatoid arthritis (RA), the synovial fluid (SF) of patients with RA (RA-SF samples), and the SF of patients without RA (non-RA-SF samples). An enzyme-linked immunosorbent assay was used to determine IgG and IgA antibody levels in 116 serum samples from patients with RA, 52 RA-SF samples, and 43 non-RA-SF samples; and these were compared with those in SF samples from 9 patients with osteoarthritis (OA-SF samples) and the blood from 100 donors (the control [CTR] group). Higher levels of IgG antibodies against *B. forsythus* (*P* < 0.0001) and *P. intermedia* (*P* < 0.0001) were found in non-RA-SF samples than in OA-SF samples, and higher levels of IgG antibodies against *B. forsythus* (*P* = 0.003) and *P. intermedia* (*P* = 0.024) were found in RA-SF samples than in OA-SF samples. Significantly higher levels of IgA antibodies against *B. forsythus* were demonstrated in both RA-SF and non-RA-SF samples than in OA-SF samples. When corrected for total Ig levels, levels of IgG antibody against *B. forsythus* were elevated in RA-SF and non-RA-SF samples compared to those in OA-SF samples. Lower levels of IgG antibodies against *B. forsythus* were found in the sera of patients with RA than in the plasma of the CTR group (*P* = 0.0003) and IgA (*P* = 0.0001). When corrected for total Ig levels, the levels of IgG and IgA antibodies against *B. forsythus* were still found to be lower in the sera from patients with RA than in the plasma of the CTR group (*P* = 0.0001). The levels of antibodies against *P. gingivalis* and *C. albicans* in the sera and SF of RA and non-RA patients were comparable to those found in the respective controls. The levels of IgG and IgA antibodies against *B. forsythus* were elevated in SF from patients with RA and non-RA-SF samples compared to those in OA-SF samples. Significantly lower levels of IgG and IgA antibodies against *B. forsythus* were found in the sera of patients with RA than in the plasma of the CTR group. This indicates the presence of an active antibody response in synovial tissue and illustrates a potential connection between periodontal and joint diseases.

Sufficient data are available to implicate *Porphyromonas gingivalis,* *Bacteroides forsythus,* and *Prevotella intermedia* as pathogens that initiate periodontal disease (2, 40, 43). These gram-negative anaerobic bacteria possess various antigens (32, 36) that provoke a host-mediated immune response to the offending species (2, 36). This is a complex immunopathogenic process which involves interactions between T and B lymphocytes, neutrophils, monocytes, and phagocytes and the subsequent production of cytokines and prostaglandins (15). The humoral immune response, in which immunoglobulin G (IgG) and IgA antibodies are produced, is considered to have a protective role in the pathogenesis of periodontal disease (2, 13, 22), but the mechanisms are not fully understood.

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by synovial hyperplasia and chronic inflammation (14). Peripheral joint disease is the most frequent feature of RA; but the eyes, skin, blood vessels, kidneys, and nervous system may also be involved. The prevalence of RA in the Western population is 0.5 to 1% and affects women about three times as often as it affects men (35). Experimental evidence has suggested that several microorganisms (bacterial proteins or viruses) may play an important role, in association with a genetic predisposition (3), in triggering the onset of the disease (25, 31, 44).

Clinical studies of RA and periodontal disease have provided evidence for a significant association between the two disorders (29). Patients with long-standing active RA have a substantially increased frequency of periodontal disease compared to that among healthy subjects (21). Patients with periodontal disease have a higher prevalence of RA than patients without periodontitis (28), and it may be hypothesized that periodontal disease plays a role as a triggering factor for RA.

Dry eyes and dry mouth have been shown to be major complaints among RA patients, but the prevalence is uncertain (4, 10, 11, 38). Decreased salivary secretion also affects the oral mucosa and may promote oral candidiasis (18). Interestingly, it has been shown that RA patients may have higher counts of oral *Candida* species than controls (19).

The last few decades of periodontal research have provided evidence for a correlation between elevated concentrations of antibodies against periodontal pathogens in serum and disease severity (2, 13, 22, 42). However, few studies that have attempted to search for a link between oral microorganisms and immune responses in the sera of patients with RA (RA sera)
TABLE 1. Levels of IgG antibodies against P. gingivalis, P. intermedia, and B. forsythus in RA and CTR group sera

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of samples</th>
<th>Mean (SD) IgG level (μg/ml)</th>
<th>Mean (SD) total IgG level (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA serum</td>
<td>116</td>
<td>9.85 (12.08)</td>
<td>2.21 (1.72)</td>
</tr>
<tr>
<td>CTR</td>
<td>100</td>
<td>7.70 (9.99)</td>
<td>1.91 (1.10)</td>
</tr>
</tbody>
</table>

*P < 0.01 for differences in antibody levels between the patient group (RA serum) and the CTR group (CTR plasma) calculated by the Mann-Whitney U test.

**P < 0.0001 for differences in antibody levels between the patient group and the CTR group calculated by the Mann-Whitney U test.

and synovial fluid (SF) samples from patients with RA (RA-SF samples) have been carried out. In a study of cell wall components from the periodontal pathogen Actinobacillus actinomycetemcomitans, it was shown that IgG antibody titers were significantly higher in RA sera than in the sera of healthy controls (41). Other findings suggest that while the levels of IgG antibodies against P. gingivalis were significantly elevated in sera from patients with periodontal disease, comparable levels were found in the sera of healthy controls and RA patients (42).

In order to further elucidate the role of periopathogens in RA, the purpose of this study was to screen for the levels of IgG and IgA antibodies against P. gingivalis, P. intermedia, and B. forsythus, and Candida albicans in serum and RA-SF samples and samples from other arthritis patients compared to those in patients with osteoarthritis (OA) and healthy subjects.

MATERIALS AND METHODS

Patients and sampling. All rheumatic patients were examined at the Rheumatology Clinic at Haukeland University Hospital, Bergen, Norway. A medical history was taken from each patient. Recorded serological data included rheumatoid factor (RF) titers and C-reactive protein (CRP) levels. CRP levels were determined by endpoint analysis (Tina Quant method) at a central laboratory, Haukeland University Hospital, during hospitalization. The cutoff level for a positive RF titer was set as a titer of 128 (11, 39), as determined by the hemagglutination method. The study was approved by the Ethical Committee, Faculty of Medicine, University of Bergen.

Group 1 consisted of 116 patients with RA. Eighty-two (70.7%) of these patients were women, and 34 (29.3%) were men. The mean ages were 58.6 years (range, 33 to 80 years) for women and 60.4 years (range, 33 to 80 years) for men. Ninety-two (79.3%) were treated with disease-modifying antirheumatic drugs (DMARDs), and the most frequent drug administered was methotrexate (MTX), which was administered to 63 patients. Steroids were given orally to 42 patients. One OA patient who provided an SF sample and three DMARDs, and 14 of them were treated with MTX. Steroids were administered orally to 22 patients. One OA patient who provided an SF sample and three non-RA patients who provided an SF sample were treated with MTX. Steroids were given orally to four of the non-RA patients who provided an SF sample. All RA patients fulfilled the revised criteria of the American College of Rheumatology (formerly the American Rheumatism Association) (7). Peripheral blood samples were collected from RA patients in group 1 at the time of medical examination and were immediately centrifuged, and the sera were stored at −20°C. SF, mainly from larger joints, was obtained only from patients in group 2, centrifuged, and stored at −20°C.

Group 3 consisted of 100 healthy blood donors (71 women, 29 men) who were matched by gender and age with the patients in group 1 and who had no history of active rheumatic disease. The mean ages were 60.4 years (range, 41 to 70 years) for women and 62.3 years (range, 33 to 80 years) for men. The plasma was stored at −20°C and served as the control (CTR) group. In this study blood donor plasma was found to fulfill the criteria as controls for RA sera.

Microbial antigens used in enzyme-linked immunosorbent assay (ELISA). P. gingivalis ATCC 33277 and P. intermedia ATCC 25611 were obtained from the American Type Culture Collection (ATCC), Manassas, Va. Both were cultured in Lab (90-A Fastidious Anaerobic Agar (LabM, IDG [United Kingdom] Limited, Bury, England), to which sheep blood was added. B. forsythus FDC 2008 was obtained from the Forsyth Dental Center, Boston, Mass., and was cultured in Trypticase soy agar with N-acetylmuramic acid stock solution.

Mannan (M7504) was obtained from Sigma Chemical Company, St. Louis, Mo. The powder was diluted in distilled water so that the concentration of the solution was 27.2 mg/ml.

The organisms were cultivated in an anaerobic glove box for 10 days at 35°C and then harvested and recultivated for 10 additional days. The second-generation bacterial cultures were inactivated in 1% formalin for 24 h, washed twice in phosphate-buffered saline (PBS), and centrifuged at 10,000 × g for 10 min. The inactivated whole cells were again washed and diluted in PBS to approximately 1,500 × 10^6 cells/ml of PBS solution by comparison with McFarland standards (24).

ELISAs for IgG and IgA antibody detection. The concentrations of IgG and IgA antibodies against whole-cell P. gingivalis, P. intermedia, and B. forsythus and the C. albicans polysaccharide mannan in RA sera, CTR plasma, and SF were determined by ELISA with 96-well microtiter flat-bottom high-binding plates (Costar, Cambridge, Mass.) (12, 16). All analyses were carried out in duplicate and were performed with 100 μl of solution per well.

The plates were coated overnight at 4°C with suspensions of inactivated whole bacterial cells (1,500 × 10^6 cells/ml of PBS).

For IgG antibody determinations, the suspensions were 1/100 for P. gingivalis and B. forsythus and 1/80 for P. intermedia. C. albicans mannan was diluted to 5 μg/ml in 0.05 M carbonate buffer. For IgA antibody determination, the suspensions were diluted 1/100 in PBS for P. gingivalis, P. intermedia, and B. forsythus. C. albicans mannan was diluted to 5 μg/ml in 0.05 M carbonate buffer.

All plates were washed four times with PBS–0.05% Tween 20 (PBST) and were then blocked with PBST–5% milk (L 31 skim milk; Oxoid, Basingstoke, England) for 1 h at room temperature.

For specific IgG antibody determinations, RA sera and CTR plasma were diluted 1/100 for P. gingivalis and P. intermedia, while SF was diluted 1/10 for P. gingivalis and P. intermedia. For B. forsythus and C. albicans mannan the RA sera and the CTR plasma were diluted 1/50 and SF was diluted 1/40. The RA sera, CTR plasma, and SF samples were diluted 1/10 for specific IgA antibody determinations. IgG and IgA antibody standards (3.125 to 200 μg/ml) were prepared by diluting purified human IgG (1.274 mg/ml; IgGos; Sigma) and human IgA (1 mg/ml; IgA1010; Sigma) in PBST–5% milk.

After 1.5 h at room temperature, the plates were washed four times with PBST. Peroxidase-conjugated goat anti-human IgG (A0293; Sigma) was diluted 1/2,000 in PBST–5% milk for all plates except those for P. intermedia, which were diluted 1/1,500 for RA sera and CTR plasma, and was then added to the plates. Peroxidase-conjugated goat anti-human IgA (A7032; Sigma) was diluted 1/3,000 for all plates.

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For IgG antibody determinations, the suspensions were 1/100 for P. gingivalis and B. forsythus and 1/80 for P. intermedia. C. albicans mannan was diluted to 5 μg/ml in 0.05 M carbonate buffer. For IgA antibody determination, the suspensions were diluted 1/100 in PBS for P. gingivalis, P. intermedia, and B. forsythus. C. albicans mannan was diluted to 5 μg/ml in 0.05 M carbonate buffer.

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After 1 h at room temperature the plates were washed four times and substrate was added. 1,2-Phenylenediamine dihydrochloride tablets (Dako, Glostrup, Denmark) were dissolved in water by the addition of H2O2. The reaction was stopped with 1 M H2SO4 and read at 490 nm with an E-max precision microplate reader (Molecular Devices Corporation, Sunnyvale, Calif.). The software SOFTmax (Molecular Devices Corporation) was used to calculate the values. Prior to all assays, pilot studies were performed with a limited number of randomized selected samples to find the most appropriate dilutions.

Total IgG and IgA levels were determined by coating the plates with goat anti-human IgG (1 mg/ml; I3382; Sigma) and goat anti-human IgA (3 mg/ml; I1261; Sigma) diluted 1/2,000 and 1/4,000 in PBS, respectively. IgG and IgA antibody standards (3.125 to 200 ng/ml) were prepared by purifying human IgG (1.274 mg/ml; I4506; Sigma) and human IgA (1 mg/ml; I1010; Sigma) in PBST-5% milk. The RA serum, CTR plasma, and SF samples were diluted 1/10 in PBST-5% milk. Peroxidase-conjugated goat anti-human IgG (A0293; Sigma) and peroxidase-conjugated goat anti-human IgA (A7032; Sigma) were diluted 1/2,000 and 1/3,000 in PBST-5% milk, respectively. The ELISA was performed as described above for the bacterial suspensions.

Calculation of antibody standard. The plates were coated with the whole bacterial suspensions. After some of the sera were screened, one sample with high levels of IgG antibodies (human serum) was used as the reference standard. This serum sample was assigned a value of arbitrary units by endpoint titration. In the subsequent analysis, twofold serial dilutions of this serum sample were added. Some other wells of the same plate were coated with bacteria, and then anti-human IgG (1 mg/ml; I3382; Sigma), and then a known amount (in nanograms per milliliter) of purified human IgG (1.274 mg/ml; I4506; Sigma) was added. Some other wells of the same plate were coated with bacteria, and then the reference serum sample was added. The same amount of peroxidase-conjugated goat anti-human IgG (A0293; Sigma) was added to the plate. The IgG titers for the human serum was read against the known IgG values. The units were translated to nanograms per milliliter.

Antibody specificity. The human serum was mixed with P. gingivalis cells (1 part diluted serum [1:50] and two parts P. gingivalis [1.5 × 10⁴ cells/ml]). The mixture was shaken on a Schützler MTS 4 shaker (IKA) for 4 h at room temperature and was then left overnight at room temperature. After centrifugation the supernatant was tested by ELISA with P. gingivalis, and B. forsythus as antigens. Anti-human IgG (I3382; Sigma) served as coat for standard IgG (I4506; Sigma). ELISA was performed as described above with peroxidase-conjugated goat anti-human IgG (A0293; Sigma).

Statistical analysis. Statistical analysis of the differences between the patient groups and the controls was carried out by the Mann-Whitney U test. The level of significance was set at a P value of < 0.05. The associations between specific antibody levels and total IgG and IgA levels were analyzed by use of Spearman’s rho correlation coefficient. The statistical analyses were performed with SPSS software (release 10.0.0; SPSS Inc., Chicago, Ill.).

### TABLE 2. Specific levels of IgG antibodies against P. gingivalis, P. intermedia, and B. forsythus in SF

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of samples</th>
<th>Mean (SD) IgG level (μg/ml)</th>
<th>P. gingivalis</th>
<th>P. intermedia</th>
<th>B. forsythus</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA-SF</td>
<td>52</td>
<td>1.43 (1.08)</td>
<td>0.82* (0.525)</td>
<td>1.51† (1.309)</td>
<td>8.80‡ (4.18)</td>
</tr>
<tr>
<td>Non-RA-SF</td>
<td>43</td>
<td>1.56 (0.641)</td>
<td>0.96* (0.401)</td>
<td>1.68 (1.156)</td>
<td>11.61* (7.44)</td>
</tr>
<tr>
<td>OA-SF</td>
<td>9</td>
<td>1.36 (0.519)</td>
<td>0.47 (0.056)</td>
<td>0.45 (0.118)</td>
<td>4.22 (1.13)</td>
</tr>
</tbody>
</table>

a * P < 0.05 for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.

b * P < 0.01 for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.

c * P < 0.0001 for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.

### RESULTS

**Total Ig levels.** The total IgG antibody levels were significantly higher in RA sera than in CTR plasma (P < 0.0001) (Table 1). Total IgG levels were also higher in RA-SF samples (P = 0.001) and SF samples from non-RA patients (non-RA-SF samples) (P < 0.0001) than in SF samples from patients with OA (OA-SF samples) (Table 2). No differences in total IgA antibody levels in sera were seen (Table 3), but significantly higher IgA antibody levels were found in RA-SF (P = 0.001) and non-RA-SF (P < 0.0001) samples than in OA-SF samples (Table 4).

**Specific IgG and IgA antibody levels.** RA sera contained significantly lower levels of antibodies against B. forsythus than CTR plasma (Tables 1 and 3). The P values for differences in IgG antibody levels were 0.003 and <0.0001 for IgA, respectively. In contrast, the levels of IgG antibodies against B. forsythus were significantly higher in both RA-SF (P = 0.003) and non-RA-SF (P < 0.0001) samples than in OA-SF samples (Table 3). Significantly higher levels of IgA antibodies against B. forsythus were also seen in RA-SF (P = 0.027) and non-RA-SF (P < 0.0001) samples than in OA-SF samples (Table 4).

The levels of IgG antibodies against P. intermedia were significantly higher in both RA-SF (P = 0.024) and non-RA-SF (P < 0.0001) samples than in OA-SF samples (Table 2). Similarly, non-RA-SF samples had stronger antibody responses against B. forsythus and P. intermedia than RA-SF samples.

The levels of IgA antibodies against P. intermedia in RA sera and SF samples and against P. gingivalis in SF samples were below the lowest standard value (<3.125 ng/ml) and were excluded as a result of the findings from the pilot studies with five randomly selected samples (Tables 3 and 4). Although the levels of IgG and IgA antibodies against P. gingivalis tended to be higher in RA serum and SF samples than in the plasma from the CTR group, the differences were not statistically significant (Tables 1 to 3).

### TABLE 3. Levels of specific IgA antibodies against P. gingivalis, P. intermedia, and B. forsythus in RA and CTR sera

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of samples</th>
<th>Mean (SD) IgA level (mg/ml)</th>
<th>P. gingivalis</th>
<th>P. intermedia</th>
<th>B. forsythus</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA-serum</td>
<td>116</td>
<td>0.169 (0.176)</td>
<td>Not detectable</td>
<td>0.349* (0.275)</td>
<td>2.33 (1.50)</td>
</tr>
<tr>
<td>CTR</td>
<td>100</td>
<td>0.129 (0.121)</td>
<td>Not detectable</td>
<td>0.532 (0.390)</td>
<td>2.28 (2.06)</td>
</tr>
</tbody>
</table>

a * Not detectable, values less than the lowest standard value used.

b * P < 0.0001 for differences in antibody levels between the patient group (RA serum) and the CTR group (CTR plasma) calculated by the Mann-Whitney U test.
Higher levels of IgG antibodies against *C. albicans* mannan were seen in RA sera than in CTR plasma, although these differences were not significant (*P* = 0.072). The differences in IgG antibody levels in RA-SF and non-RA-SF samples compared to those in OA-SF samples were not statistically significant, and the detectable IgA levels were below the lowest standard value.

**Total Ig and specific antibody levels.** Significant associations between specific antibodies and the total Ig levels in serum and SF samples were detected. In RA serum (Fig. 1 and 2), total IgG antibody levels correlated with the levels of IgG antibodies against *P. intermedia* (*r* = 0.251; *P* = 0.007) and *B. forsythus* (*r* = 0.249; *P* = 0.007). No significant correlation was found between the levels of IgG antibodies against *P. gingivalis* and the total IgG level in RA sera.

A significant correlation between the total IgA antibody level and the *B. forsythus*-specific IgA level was evident in RA serum (*r* = 0.198; *P* = 0.033).

A significant correlation between the levels of IgG antibodies against *P. intermedia* and total IgG levels in RA-SF samples was present (*r* = 0.519; *P* < 0.0001) (Fig. 3). Increased levels of IgG (*r* = 0.372; *P* = 0.007) and IgA (*r* = 0.588; *P* < 0.0001) antibodies against *B. forsythus* correlated with increased total IgG and IgA antibody levels in RA-SF samples. No significant relation between the total antibody levels and specific Ig antibody levels in non-RA-SF samples was shown. Significant relations between total antibody levels and *C. albicans* mannan specific antibodies were absent for RA serum, CTR plasma, and SF samples.

When corrected for the total Ig levels (Ig level/total Ig level), lower levels of IgG and IgA antibodies against *B. forsythus* in RA serum than in CTR plasma (*P* < 0.0001) were demonstrated (Table 5). Although the differences were not significant (*P* = 0.120), a tendency toward higher levels of IgG antibodies against *B. forsythus* in the non-RA-SF and RA-SF samples than in OA-SF samples was seen. For IgA this tendency was also evident in non-RA-SF samples (Table 5). Minor differences were detected for *P. intermedia*.

**Serological data and antibody levels.** The sera of 63 (54.3%) of the RA patients were RF positive, and significant relations between RF titers and total IgA antibody levels (*r* = 0.698; *P* < 0.0001) and between RF titers and the levels of IgG antibodies against *P. gingivalis* (*r* = 0.189; *P* = 0.043) were found. No significant associations between RF titers and total IgG levels or between RF levels and the levels of specific Ig against the other bacteria and *C. albicans* were seen.

The SF of 16 (32%) of the 50 RA patients was RF positive, and a significant correlation between RF levels and total IgA antibody levels was found (*r* = 0.449; *P* = 0.001). The SF of all of the non-RA patients was RF negative, and the SF of one of

### Table 4. Levels of IgA antibodies against *P. gingivalis*, *P. intermedia*, and *B. forsythus* in SF

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of samples</th>
<th>Mean (SD) IgA level (µg/ml)</th>
<th>Mean (SD) total IgA level (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>P. gingivalis</em></td>
<td><em>P. intermedia</em></td>
</tr>
<tr>
<td>RA-SF</td>
<td>52</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Non-RA-SF</td>
<td>43</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
<tr>
<td>OA-SF</td>
<td>9</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>

*Not detectable, values less than the lowest standard value used.*

*P* < 0.05 for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.

*P* < 0.01 for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.

*P* < 0.0001 for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.

![Total IgG (mg/ml) vs. Anti- *P. intermedia*-IgG (µg/ml)](image)

**FIG. 1.** Relation between levels of IgG antibodies against *P. intermedia* and total IgG levels in RA serum.
the OA patients was RF positive. No significant correlation was seen between RF titers and total IgG antibody levels or between RF levels and the levels of Ig against the other bacteria and *C. albicans* in SF.

The median CRP level among the serum samples from RA patients was 20.8 mg/liter (range, 0 to 139 mg/liter). A significant association between CRP and total IgG levels \( (r = 0.291; P = 0.002) \) was found. Positive associations between CRP levels and both the levels of IgG antibodies against *B. forsythus* \( (r = 0.187; P = 0.047) \) and the levels of IgG antibodies against *P. intermedia* \( (r = 0.227; P = 0.016) \) were also demonstrated.

The mean CRP level for the RA-SF group was 52.0 mg/liter (range, 2.0 to 122.0 mg/liter), for the OA-SF group was 5.8 mg/liter (range, 4.0 to 9.0 mg/liter), and for the non-RA-SF group was 43.4 mg/liter (range, 0 to 240 mg/liter). For the RA-SF group, there was a significant correlation between CRP levels and the levels of IgA antibodies against *B. forsythus* \( (r = 0.302; P = 0.037) \) and between CRP levels and total IgA levels \( (r = 0.489; P < 0.0001) \).

**Antigen specificity.** Testing of the antigen specificity between *P. gingivalis* and the other two bacteria showed that 70% of the anti-*P. gingivalis* IgG antibodies did not react when they were mixed with the *P. intermedia* suspension and that 77% of the anti-*P. gingivalis* IgG antibodies did not react when they were mixed with the *B. forsythus* suspension.

**DISCUSSION**

In this study we have shown that there is a different immune response against some selected periodontal pathogens in se-
rum and SF between arthritis patients and controls. Significantly higher levels of IgG and IgA antibodies against *B. forsythus* and *P. intermedia* were found in SF from RA- and non-RA-patients than in SF from OA patients. However, the levels of antibodies against *P. gingivalis* in RA sera were comparable to those found in the plasma of the CTR group, which has also been demonstrated by others (42). These findings could indicate that a stronger activation of specific B cells with the subsequent formation of antibodies directed especially against *B. forsythus* and *P. intermedia* is present in the joints of RA patients and patients with other arthritides.

The lower total antibody levels in SF compared to those in serum, findings which are in agreement with those of other investigators (27), imply that this immune response predominately takes place in the surrounding tissue and to a lesser degree in the joint cavity. A possible mechanism for this is the capture of bacterial DNA or bacterial products in the synovial tissue, where they promote proinflammatory responses. Plasma cells may also be recruited nonspecifically to the joint. Higher mean levels of Ig antibodies (Tables 1 to 5) against *B. forsythus* compared to the levels of antibodies against *P. intermedia* were found. This and the fact that only IgA antibodies against *B. forsythus* could be detected in SF support the assumption of a particularly strong synovial immune response to *B. forsythus*.

We also demonstrated significantly lower levels of IgG and IgA antibodies against *B. forsythus* in RA serum than in the plasma of the CTR group. Given the higher SF antibody concentrations, it could be assumed that the B-cell homing mechanism toward synovial tissue is more pronounced in RA patients and patients with other arthritides and that this homing mechanism is particularly strengthened for *B. forsythus*. It has also been demonstrated that mucosal lymphocytes in particular are able to home to the synovium by expression of relevant adhesion molecules (8). The explanation why the levels of antibodies against this pathogen were particularly lower in RA serum is disputable. However, studies have shown reduced systemic levels of IgG against peptidoglycans from intestinal bacteria, indicating insufficient protection from spreading peptidoglycans, which was hypothesized to contribute to inflammatory processes (30).

To the best of our knowledge, our study is the first to investigate the role of periodontal pathogens in SF from RA patients and patients with other arthritides. Previous studies have focused on the role of antibody levels in the sera of patients with periodontal disease (2, 13, 22, 42). Researchers have also been interested in the role of the concentrations of antibodies against periodontal pathogens in the serum of RA patients and have investigated the relationship between periodontal disease and RA (21, 28, 29, 33, 41, 42). However, very few studies have been able to provide clinical periodontal data as a supplement to the immune status. Periodontal status has been shown to vary between RA patients and controls, and it is assumed that patients with long-standing active RA have an increased frequency of periodontal disease (21). Our findings regarding the mean antibody levels (Tables 1 to 5) could support this assumption that the incidence of periodontal disease could be higher in RA patients and patients with other arthritides than in healthy blood donors and OA patients, respectively.

Many similarities between the immunopathologies of RA and periodontal disease have been detected (29, 41, 42). Both are destructive, chronic, and inflammatory conditions and may have an acute or chronic presentation. Periodontitis affects single or multiple sites within the oral cavity, leading to the loss of the supporting periodontal tissue (23). In parallel with the pathogenesis seen in RA, the degenerative capability of periodontitis may be initiated and maintained by the presence of endotoxins and exotoxins introduced by microbial organisms (13, 32, 44). It is suggested that while T lymphocytes and polymorphonuclear cells dominate in the early stages of plaque accumulation and gingivitis, B cells dominate in more advanced lesions, with a higher degree of soft tissue destruction and bone loss (9, 36). As is seen in periodontal disease (5, 15, 20, 36), B cells are activated in the synovial tissue in patients with arthritis, leading to a spontaneous secretion of Igs (IgG, IgA, and IgM) (1). This lymphocytic response may lead to the subsequent formation of immune complexes, which are capable of inducing a spectrum of inflammatory mediators (17, 34). In patients with periodontal disease, inflammatory mediators are connected to tissue destruction and bone loss (15).

The cell wall polysaccharide mannan is considered a major antigen of the yeast *C. albicans* and has the ability to stimulate specific cell-mediated and humoral IgG and IgA immune responses (26). It has been shown that mannan antibodies more frequently appear in sera from patients with Crohn’s disease and ulcerative colitis than in controls (6). In our study, we did not find any significant differences in seroreactivity to *C. albi-

### TABLE 5. Levels of antibodies against *B. forsythus* and *P. intermedia* corrected for total Ig levels

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of samples</th>
<th>Mean (SD) ratio (10^5) of IgG level/total IgG level</th>
<th>Mean (SD) ratio (10^5) of IgA level/total IgA level</th>
</tr>
</thead>
</table>
|              |                | *B. forsythus* | *P. intermedia* |                               | *B. forsythus* | *P. intermedia*
| RA serum     | 116            | 15.7^b (12.4) | 8.90^b (5.5)    | 20.6^b (23.0) | Not detectable |
| Control plasma| 100           | 28.3 (23.2)   | 12.4 (11.1)     | Not detectable | Not detectable |
| RA-SF        | 52             | 18.3 (14.4)   | 10.3 (6.7)      | 22.2^b (16.6) | Not detectable |
| Non-RA-SF    | 43             | 19.7 (17.5)   | 11.3 (7.9)      | 40.1 (39.5)   | Not detectable |
| OA-SF        | 9              | 11.7 (5.0)    | 11.8 (5.9)      | 32.4 (12.8)   | Not detectable |

^a P < 0.0001 for differences in antibody levels between the patient group (RA serum) and the CTR group (CTR plasma) and between RA-SF, non-RA-SF, and OA-SF samples calculated by the Mann-Whitney U test.

^b P < 0.05 for differences in antibody levels between the patient group (RA serum) and the CTR group (CTR plasma) and between RA-SF, non-RA-SF, and OA-SF samples calculated by the Mann-Whitney U test.

^c Not detectable, values less than the lowest standard value used.
between related species in the oral and the intestinal in other patient groups. This could be due to earlier exposure ies against these bacteria in arthritis patients demonstrate that oral microbial might assume that our results elucidate an association between rial DNA has been presented (30, 37). In light of this, one connection between arthritis and bacterial products or bacte- those in the OA-SF samples.


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