Evidence for differential effects of sulphasalazine on systemic and mucosal immunity in rheumatoid arthritis

Lars Kanerud, Gunilla Norhagen Engström, Andrej Tarkowski

Abstract

Objective—To study the effects of sulphasalazine (SASP) on the systemic and mucosal humoral immune systems in patients with rheumatoid arthritis (RA).

Methods—Serum concentrations of interleukin 6 (IL-6), class and subclass specific IgG, IgA and IgM, IgA and IgG anti-gliadin antibodies and rheumatoid factors (RF) of IgG, IgA (including IgA1 and IgA2 subclasses) and IgM isotypes were measured before and 16 weeks after sulphasalazine (SASP) therapy in 15 female and three male patients with RA. Amounts of immunoglobulins in saliva and jejunal fluid were measured as estimates of mucosal humoral immunity.

Results—Serum concentrations of IgA and IgG decreased significantly during SASP therapy and correlated with reduced concentrations of IL-6. In addition, levels of circulating IgA RF, IgA anti-gliadin antibodies and IgM RF decreased significantly after the treatment. In contrast, immunoglobulin levels in saliva and jejunal fluid were unaltered.

Conclusion—SASP exerts powerful but selective inhibitory effects on systemic immunoglobulin production, whereas no effects on mucosal immunoglobulin production were observed. The decreased systemic B cell activity may be mediated by downregulation of the production of IL-6, a cytokine with Ig switching properties.

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Sulphasalazine (SASP) has been shown to be an effective agent for the treatment of rheumatoid arthritis (RA), although the mode of action by which it reduces disease activity is unclear. SASP has been reported to reduce neutrophil function^{1 2} in addition to possessing immunomodulatory effects,³⁻⁶ but may also induce a state of humoral immunodeficiency.⁷⁻⁹

Because SASP also displays potent beneficial properties in inflammatory bowel disease, interest has been focused on the possibility that the effect of SASP on RA may be mediated by modulation of intestinal immunity.^{10 11} The rationale for this hypothesis includes the proposal of an enteric cause of RA,¹² findings that certain dietary antigens exacerbate symptoms in RA,¹³ and more recent obser-

vations that some patients with RA display ileocaecal inflammation.¹⁴ SASP has been shown to decrease intestinal inflammation in RA patients.¹⁵

The mucosal immune system is considered to be the major immunoglobulin forming organ in the human body.¹⁶ The intestinal immunocytes produce mainly secretory IgA (sIgA),¹⁷ and are frequently triggered by orally delivered antigens.^{18 19} Occurrence of IgA rheumatoid factor (RF) has been linked to RA with systemic manifestations, an erosive disease course, or a severe disease outcome.²⁰⁻²³ IgA RF has been shown to be produced in the mucosal compartments,^{24 25} and is suggested to be a marker of inflammation in or near mucosal membranes.²⁶ Activation of the mucosal immune system should therefore be considered in the pathogenesis of RA.

To study in vivo if the administration of SASP affected humoral immunity in the gastrointestinal tract and peripheral blood in RA patients, we prospectively analysed the concentrations of major classes and subclasses of immunoglobulins in serum, saliva, and jejunal fluid, in addition to the serum levels and isotypic distribution of RF and anti-gliadin antibodies before and after 16 weeks of treatment with sulphasalazine.

Patient and methods

We studied 21 patients (17 female) with clinically active RA, fulfilling the criteria of the American Rheumatism Association for the diagnosis of classical or definite RA.²⁷ Previous records showed that the patients were all seropositive for rheumatoid factor. Apart from their RA, the patients were healthy and none complained of gastrointestinal symptoms. Their median age was 54 years (range 24-71) and the median duration of the disease was 73 months (range 6-204).

Clinically active disease was defined as the presence of at least two of the following three criteria: duration of morning stiffness ≥ 60 minutes, tenderness, swelling, or both, of at least six joints, and Westergren erythrocyte sedimentation rate (ESR) ≥ 30 mm/1st h.

None of the patients had received treatment with disease modifying antirheumatic drugs or corticosteroids for at least three months before inclusion in the study.

All patients had given their informed consent before the study, which was approved by the local Ethics Committee.

Department of Rheumatology, Karolinska Institute, Stockholm Söder Hospital, Sweden
L Kanerud

Department of Immunology, Microbiology, Pathology and Infectious Diseases, Huddinge University Hospital, Huddinge, Sweden
G N Engström

Department of Rheumatology and Clinical Immunology, Gothenburg University, Sweden
A Tarkowski

Correspondence to: Dr Lars Kanerud, Department of Rheumatology, Stockholm Söder Hospital, S-118 83 Stockholm, Sweden.

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STUDY DESIGN

The patients were recruited for a larger study of the gut in RA and the effects of SASP, reported in part elsewhere,²⁸ and were treated with SASP 1.5–3 g/day. Before treatment was commenced and 16 weeks thereafter, peripheral blood was drawn and analysed for contents of immunoglobulins (major classes and subclasses), rheumatoid factors, anti-gliadin antibodies, acute phase reactants, and interleukin 6 (IL-6). Saliva and jejunal fluid for immunoglobulin analysis were collected in association with duodenal-jejunal mucosal biopsy sampling at the same times, and disease activity was assessed with an index based on Lansbury's joint index, the duration of morning stiffness, and the ESR.²⁸ All samples were collected between 08:00 and 10:00 after an overnight fast.

CLASS AND SUBCLASS SPECIFIC

DETERMINATIONS OF SERUM

IMMUNOGLOBULINS

Serum concentrations of IgG, IgA, IgM, and orosomucoid were determined by turbidimetry on a Multistat III centrifugal analyser (Instrumentation Laboratory Ltd, Warrington, UK), using antiserum from Dakopatts (Copenhagen, Denmark) and protein calibrators from Behringwerke (Marburg, Germany). The reference values for IgG, IgA, IgM, and orosomucoid were 7.0–15.0, 0.7–3.5, 0.2–2.2, and 0.4–1.1 g/l, respectively.

IgG subclass concentrations were determined according to routine methods using Mancini immunodiffusion. The reference values for IgG1, IgG2, IgG3, and IgG4 were 4.22–12.9, 1.17–7.47, 0.41–1.29, and 0.00–2.91 g/l, respectively.

Total concentrations of serum IgA subclasses were determined by enzyme linked immunosorbent assay (ELISA) as previously described.²⁹ Briefly, serial dilutions of IgA1 (Pet) and IgA2 (Fel) purified myeloma standards (a generous gift from Dr Jiri Mestecky, Birmingham, AL, USA) and serum samples, all in phosphate buffered saline (PBS)-10% fetal calf serum (FCS), were incubated overnight in 96 well polystyrene microtitre plates (Dynatech, Alexandria, VA, USA) coated with 5 µg/ml monoclonal anti-IgA1 or anti-IgA2 (Nordic Laboratories, Tilburg, Netherlands) and blocked with PBS-FCS. After incubation and washing, biotin conjugated F(ab')₂ fragments of affinity purified goat anti-human IgA (Tago, Burlingame, CA, USA) diluted 1:1500 in PBS-Tween containing 10% FCS were added to the microtitre plates, followed by incubation with 1 µg/ml of horseradish peroxidase labelled avidin (Sigma Chemical Company, St Louis, MO, USA) and, finally, the enzyme substrate 2,2 azino-bis (3-ethyl-benzthiazoline-6-sulphonic acid) (Sigma) in citrate buffer, pH 4.2, containing 0.0075% hydrogen peroxide (H₂O₂). The absorbance was measured in a Titertek Multiscan photometer (Flow, McLean, VA, USA) at 414 nm. All samples were run in duplicate. Optical density (OD)

values were converted to g/l using calibration curves based on the OD obtained from serial dilutions of the respective highly purified IgA myeloma proteins. Calibration curves were constructed using a computer program based on the weighted logit-log model.³⁰

DETERMINATION OF SERUM IL-6 LEVELS

Serum levels of IL-6 were determined by a bioassay as previously described.³¹ Briefly, we used B9 subclone³² originating from cell line B 13-29, which is dependent on IL-6 for growth. B9 cells were harvested from tissue culture flasks, seeded into microtitre plates (Nunc, Roskilde, Denmark) at a concentration of 5000 cells per well, and cultured in Isocove's medium supplemented with 5×10^{-5} mol/l 2-mercaptoethanol, 5% FCS, and 50 µg/ml gentamycin. Heat inactivated serially diluted serum samples and recombinant human IL-6 standard (Genzyme, Cambridge, MA, USA) were added to the wells. 3H-Thymidine was added after 68 hours of culturing, and the cells were harvested four hours later. All samples were run in triplicate. The sensitivity limit for this assay is below 1 pg/ml. B9 cells were previously shown not to react with several recombinant cytokines, including IL-1α and β, IL-2, IL-3, IL-5, granulocyte macrophage colony stimulating factor, tumour necrosis factor α and interferon gamma. There was only weak reactivity with IL-4.³³

SALIVA AND JEJUNAL ASPIRATE

Unstimulated whole saliva was collected in the morning after overnight fasting by direct expectoration into a sterile plastic container and was kept at -70°C until required for analysis. In order to obtain jejunal aspirate with a minimum of microbial contamination from the stomach and oral cavity, a special closed tube system (RHP-12-0, William Cook Europe AB, Bjaeverskov, Denmark) was used, as described earlier.³⁴ The tube system was attached to the biopsy collecting device during intubation and clearly visualised fluoroscopically. Aspirate was collected from the proximal part of jejunum immediately before biopsy sampling and thereafter kept in plastic tubes at -70°C until analysis.

DETERMINATION OF IMMUNOGLOBULINS IN

SALIVA AND JEJUNAL ASPIRATE

The saliva and jejunal samples were thawed and centrifuged for 15 minutes at 10 000 g at 4°C and the supernatant assayed by ELISA.³⁵ Each sample of saliva and jejunal fluid was tested in duplicate at three fivefold dilution steps. All saliva and jejunal fluid samples were diluted in PBS containing 0.05% Tween 20 (Polysorbatum 20, Apoteksbolaget, Sweden) (PBS-Tween). Polystyrene microtitre plates (M 129B, Dynatech) were coated overnight with anti-immunoglobulins at room temperature and stored at 4°C until used. Each well was coated with 100 µl of rabbit immunoglobulins directed toward human IgM

(2.55 µg/ml) (µ specific, Dakopatts), IgG (2.85 µg/ml) (γ specific, Dakopatts), IgA (1.5 µg/ml) (α specific, Dakopatts), or secretory component (0.85 µg/ml) (Dakopatts) in 0.1 mol/l carbonate-bicarbonate buffer (pH 9.6). The microtitre plates coated with antibodies against secretory component were blocked with 100 µl bovine serum albumin (Sigma), 5 mg/ml, for four hours at room temperature. Saliva and jejunal samples, diluted 1:1250, 1:6250, 1:31250 for sIgA and IgA, and 1:250, 1:1250, 1:6250 for IgG and IgM, were added to the microtitre plate wells in a volume of 100 µl and incubated overnight at room temperature.

Between the various incubation steps, the wells were rinsed five times with saline containing 0.05% Tween 20 (saline-Tween). Alkaline phosphatase (ALP) conjugated immunoglobulin fractions (100 µl) of mono-specific rabbit antisera to human IgM, IgG, and IgA (Dakopatts) diluted 1:1000 in saline-Tween were added and incubated for three hours at room temperature. After the final wash, disodium p-nitrophenyl phosphatase, 1 mg/ml (Sigma) in 10% diethanolamine buffer was added and the absorbance measured at 405 nm at 10 minute intervals.

Colostrum was collected from five individuals and pooled. The purification of IgA from colostrum was performed by removing IgG and IgM by affinity chromatography using cyanogen bromide (CNBr)-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). The contamination by IgM and IgG was below the background value and could not be detected by ELISA. The purified colostrum IgA was used as a reference standard for quantitation of sIgA and IgA. For measurements of IgG and IgM, standard human serum (Lot 041015 F, Behringwerke) served as standard. All standards were diluted in PBS-Tween in sixfold dilution steps. Duplicate samples of 100 µl each were used.

DETERMINATION OF SERUM ANTI-GLIADIN ANTIBODIES

Anti-gliadin antibodies were assayed with a solid phase ELISA.³⁶ Microtitre plates were coated with 50 µg/ml gliadin (Sigma) dissolved in 70% ethanol. The plates were incubated for 60 minutes with serum diluted 1:10 in PBS containing 0.5% human serum albumin, and then washed. ALP conjugated rabbit anti-human IgG or IgA (Dakopatts) was used in appropriate dilutions. The absorbance was read spectrophotometrically. All serum samples were tested in duplicate. The reference values were ≤47 units for IgG anti-gliadin antibodies and ≤30 units for IgA anti-gliadin antibodies.

CLASS AND SUBCLASS SPECIFIC DETERMINATIONS OF RF

Levels of IgM RF and IgG RF were determined using a diffusion-in-gel ELISA (DIG-ELISA) as previously described.³⁷ Briefly, the inner surfaces of polystyrene Petri

dishes were coated with 25 µg/ml of heat aggregated rabbit IgG. Melted agar was then poured into each dish. Wells 3 mm in diameter were punched in the gel and filled with the serum samples. After incubation at room temperature for 66 hours in a humid atmosphere, the agar was removed. The respective affinity purified, biotinylated F(ab')₂ fragments of anti-human IgG or IgM (Dakopatts), diluted 1:1000 in PBS-Tween were then poured into the dishes. After a two hour incubation, the dishes were rinsed, reincubated for a further one hour with 1 µg/ml horseradish peroxidase labelled avidin (Sigma) and rinsed; finally, melted 1% agar, containing 0.05% paraphenylenediamine and 0.01% H₂O₂ was poured into each dish. Positive reactions were recognised as gradually developing, brownish, circular areas. All the plates were photographed after 15 minutes and the diameters of the coloured areas were measured with a ruler to the nearest mm. Reference levels for RF isotypes have been established in a previous study.²⁰

To assess RF levels of IgA subclasses, the DIG-ELISA assay was used, with 5 µg/ml monoclonal anti-IgA1 or anti-IgA2 (Nordic) antibodies instead of class specific reagents. After two hours incubation the dishes were rinsed and reincubated with F(ab')₂ fragments of affinity purified and biotinylated rabbit anti-mouse IgG (Dakopatts) diluted 1:1000 in PBS-Tween. All the subsequent steps were as for determination of IgG and IgM RF.

IgA RF analysis was performed using a commercial sandwich enzyme immunoassay (SELISA) (Cambridge Life Sciences, Ely, Cambridgeshire, UK), according to the instructions of the manufacturer. Horse IgG was used as antigen and anti-IgA peroxidase as conjugate. All samples were tested in duplicate. Serum samples with IgA RF concentrations <15 units/ml were considered negative according to the reference values of the laboratory.

STATISTICAL ANALYSIS

Non-parametric two tailed tests were used. Wilcoxon signed rank test was used for comparisons of variables before and after SASP therapy and Spearman's rank correlation test was used for correlation purposes. Values of $p \leq 0.05$ were considered significant. The calculations were performed using CSS: STATISTICA™ software (StatSoft, Inc, Tulsa, OK, USA).

Results

Three of the original 21 patients had to stop SASP therapy because of side effects and their data were therefore excluded from comparisons of the variables before and after therapy. In one of these three patients, all tests before therapy were performed and are included in the correlation analysis before therapy. One patient refrained from a second sampling of jejunal aspirate and one serum for analysis of IgG subclasses was lost.

DISEASE ACTIVITY

The patients responded to SASP treatment with a significant decrease in inflammatory activity measured by acute phase reactants and disease activity index.²⁸ No patients deteriorated during therapy. Disease activity index decreased from 71 (33–173) index units (median and range) before, to 27 (3–68) units after SASP treatment ($p < 0.001$). Serum concentrations of orosomucoid decreased from 1.42 (1.02–3.12) to 0.89 (0.58–1.67) g/l ($p < 0.001$) and the ESR decreased from 46 (16–90) to 16 (6–48) mm/1st h ($p < 0.001$).

SERUM IMMUNOGLOBULINS

Table 1 shows the serum concentrations of immunoglobulins and their subclasses before and after SASP therapy. Before therapy, the median value for IgA in the RA patients was increased compared with the reference values of the laboratory, while remaining immunoglobulin concentrations were within the normal range. Total IgA and total IgG and their respective subclasses all decreased although the

decrease for IgA1 did not reach statistical significance. The median concentration of IgM was unaltered by SASP. No correlations were observed between the concentration of any immunoglobulin class or subclass and age, disease activity index, serum orosomucoid, or ESR before SASP therapy. Striking correlations were observed between the reductions in total IgA and total IgG ($r = 0.86$; $p = 0.00005$), and between the reductions in IgA1 and total IgG ($r = 0.52$; $p = 0.028$).

SERUM IL-6

Serum IL-6 decreased, although not significantly, in the RA patients during SASP therapy (table 1). Because determination of small concentrations of IL-6 (< 20 pg/ml) is unreliable in our assay system, we examined the seven RA patients having original IL-6 values ≥ 20 pg/ml. In these patients, IL-6 concentration decreased from 34 (20–130) pg/ml (median and range) before therapy to 16 (range from not detectable to 20) pg/ml after therapy ($p = 0.018$). At the initiation of therapy, serum IL-6 values showed a weak correlation with serum orosomucoid ($r = 0.45$; $p = 0.05$), but not with serum concentrations of immunoglobulins or rheumatoid factors. However, the reduction in IL-6 found after SASP treatment correlated with the reduction in total serum IgA ($r = 0.56$; $p = 0.015$) and with that of serum IgG ($r = 0.46$; $p = 0.056$).

IMMUNOGLOBULINS IN SALIVA AND JEJUNAL ASPIRATES

As expected, salivary immunoglobulins were primarily IgA; this was less true for jejunal fluid (table 2). No correlations were observed between salivary and jejunal concentrations of immunoglobulin, or between salivary or jejunal immunoglobulins and disease activity measurements. Furthermore, no convincing associations between salivary or jejunal and peripheral blood immunoglobulin concentrations were seen at the initiation of therapy, except for a modest correlation between salivary IgM and serum IgM values ($r = 0.46$; $p = 0.047$). The immunoglobulin levels in saliva and jejunal fluid did not change significantly after SASP therapy.

ANTI-GLIADIN ANTIBODIES

Six of 19 patients (32%) had increased serum IgA anti-gliadin antibodies before therapy, and two of 18 (11%) after SASP therapy; none had increased IgG anti-gliadin antibodies either before or after therapy. The serum levels of IgA anti-gliadin antibodies decreased significantly during SASP therapy, whereas the decrease in IgG anti-gliadin antibodies did not reach statistical significance (table 3). Serum levels of IgA anti-gliadin antibodies correlated weakly with serum IgA before therapy ($r = 0.39$; $p = 0.095$), as did the reductions in IgA anti-gliadin antibodies and the reductions in serum IgA after SASP therapy ($r = 0.42$; $p = 0.084$). No corresponding correlation regarding IgG

Table 1 Serum concentrations of immunoglobulins, their subclasses, and IL-6 in 18 patients with RA before and after 16 weeks of SASP therapy

	Before SASP	After 16 weeks of SASP	p^*
IgA (g/l)	4.15 (1.70–5.95)	2.82 (1.20–5.57)	0.0004
IgA1 (g/l)	1.47 (0.48–4.42)	0.92 (0.41–5.55)	0.07
IgA2 (g/l)	0.26 (0.07–1.34)	0.20 (0.04–0.75)	0.03
IgG (g/l)	14.2 (8.0–22.1)	12.8 (6.6–21.9)	0.006
IgG1† (g/l)	11.3 (4.4–16.5)	9.3 (4.5–19.2)	0.02
IgG2† (g/l)	3.7 (1.5–5.6)	3.1 (0.8–5.8)	0.002
IgG3† (g/l)	0.7 (0.3–2.7)	0.6 (0.2–1.3)	0.001
IgG4† (g/l)	0.60 (ND‡–1.47)	0.36 (ND‡–1.01)	0.0007
IgM (g/l)	1.82 (0.40–4.46)	1.80 (0.54–4.02)	0.23
IL-6 (pg/ml)	16 (ND§–130)	12 (ND¶–35)	0.12

Values are median (range). *Comparison before *v* after SASP therapy (Wilcoxon signed rank test).

†n = 17. ND = Not detectable; ‡ in 1/17 patients; § in 4/18 patients; ¶ in 7/18 patients.

Table 2 Immunoglobulin concentrations in saliva and jejunal aspirates in 18 and 17 patients, respectively, with RA, before and after 16 weeks of SASP therapy

	Before SASP	After 16 weeks of SASP	p^*
Saliva (mg/l)			
Total IgA	386 (189–965)	382 (110–693)	0.10
sIgA	261 (150–831)	253 (94–565)	0.32
IgG	20 (2.6–88.0)	23 (1.4–94.5)	0.37
IgM	7.5 (1.7–29.4)	5.7 (1.1–31.6)	0.29
Jejunum (mg/l)			
Total IgA	64 (3.8–200)	40 (1.3–239)	0.16
sIgA	38 (0.4–134)	24 (0.2–122)	0.21
IgG	28 (1.0–78.0)	14 (ND‡–87.6)	0.55
IgM	38 (ND§–154)	14 (ND¶–252)	0.26

Values are median (range). *Comparison before *v* after SASP therapy (Wilcoxon signed rank test).

ND = Not detectable; ‡ in 2/17 patients; § in 1/17 patients; ¶ in 3/17 patients.

Table 3 Serum levels of IgA RF, IgA1 RF, IgA2 RF, IgG RF, IgM RF, and IgA and IgG anti-gliadin antibodies (AGA) in 18 patients with rheumatoid arthritis before and after 16 weeks of SASP therapy

	Before SASP	After 16 weeks of SASP	p^*
IgA RF (U/ml)	124 (ND–600)	50 (ND–600)	0.00098
IgA1 RF (mm)	9 (ND–14)	8 (ND–14)	0.0035
IgA2 RF (mm)	ND (ND–8)	ND (ND–9)	0.29
IgG RF (mm)	5 (ND–16)	3 (ND–14)	0.28
IgM RF (mm)	8 (ND–14)	6.5 (ND–14)	0.0019
IgA AGA (U)	20.3 (ND–165)	12.6 (ND–84.2)	0.001
IgG AGA (U)	3.0 (ND–36)	2.8 (ND–24.0)	0.07

Values are median (range). *Comparison before *v* after SASP therapy (Wilcoxon signed rank test).

ND = Not detectable.

anti-gliadin antibodies and serum IgG was observed ($r = 0.22$; $p = 0.36$ and $r = 0.27$; $p = 0.26$, respectively). IgA anti-gliadin antibodies in serum showed a weak correlation ($r = 0.50$; $p = 0.030$) with sIgA in jejunal fluid. In contrast, no correlations with sIgA levels in saliva could be demonstrated ($r = 0.22$; $p = 0.36$).

RHEUMATOID FACTORS

All patients had previously been seropositive for IgM RF according to the inclusion criteria. At the start of the present study, 18/19 (95%) were positive for IgM RF, 12/19 (63%) for IgG RF and 17/19 (89%) for IgA RF. Of those positive for IgM RF, 11/18 (61%) were also positive for IgG RF and 17/18 (94%) for IgA RF. All 11 patients who were positive for both IgM RF and IgG RF were also positive for IgA RF. The only patient negative for IgM RF was positive for IgG RF but negative for IgA RF. Regarding IgA RF subclasses, 16/19 (84%) had IgA1 RF and 5/19 (26%) IgA2 RF. All patients with IgA2 RF also had IgA1 RF. After SASP therapy, 15/18 patients (83%) were positive for IgM RF, 8/18 (44%) for IgG RF, 14/18 (78%) for IgA RF, 14/18 (78%) for IgA1 RF, and 2/18 (11%) for IgA2 RF.

Table 3 shows serum levels of RF isotypes. After SASP therapy, highly significant reductions in IgA RF, IgA1 RF, and IgM RF were noted, but only minor reductions in IgA2 RF and IgG RF.

Discussion

In the present study, SASP treatment of RA patients did not affect the amounts of IgA, sIgA, IgG or IgM produced in mucosal compartments. In contrast, the reduction in serum IgA was highly significant, as was that in serum IgG. In addition, serum IgA RF, IgM RF and IgA class anti-gliadin antibody levels decreased significantly as a consequence of SASP administration.

IgA, which is the major immunoglobulin in secretory glands and the gastrointestinal tract,¹⁷ appears mostly in a polymeric form in secretions and is synthesised locally.³⁸ In mucosal tissues, polymeric IgA (pIgA) is transported to the lumen after binding to a glycoprotein secretory component (SC) produced by and located at the basolateral cell membrane surface of epithelial cells. The pIgA-SC complexes are thereafter translocated by endocytosis to the lumen.¹⁶ Monomeric IgA (mIgA) is found in intestinal juices in small amounts, but may increase in gut diseases as a result of leakage of extravascular proteins into the lumen.³⁹ In serum, the IgA1 subclass predominates and amounts to more than 80% of IgA, whereas the IgA2 subclass accounts for up to 50% of the total IgA in secretions.⁴⁰

The median serum IgA in our RA patients was increased. Increased serum IgA occurs frequently in RA⁴¹⁻⁴⁴ and has been interpreted by some authors as reflecting a stimulated mucosal immune system.⁴⁵ However, no substantial proof for this hypothesis exists.

Serum IgA, like IgG and IgM, is mainly derived from the bone marrow, with smaller contributions from the spleen and peripheral lymph nodes,^{46, 47} and its synthesis is largely independent of sIgA concentrations. This fact, in conjunction with the observed simultaneous and significantly intercorrelated changes in serum IgA and serum IgG (but not sIgA in saliva and jejunal fluid) induced by SASP, strongly suggests that SASP affects the systemic lymphoid compartment, and hardly reflects immunomodulatory effects at the intestinal level.

The decrease in IL-6 in serum after SASP therapy was correlated with the decrease in IgA and IgG concentrations in serum. Besides inducing acute phase proteins, IL-6 is a potent B cell differentiation factor⁴⁸ and is closely involved in the regulation of IgA and IgG production. This raises the possibility that the decreased serum immunoglobulin concentrations observed during SASP treatment may depend on SASP inhibiting IL-6 production in the synovial membrane and other loci. The proposed downregulation of B cells in the systemic lymphoid compartment may also be a direct effect of the drug on B cells or regulatory T cells, or both, as shown previously in vitro.^{3, 4, 6} Alternatively, the drug might influence the reactivity of antigen presenting cells.

Previous clinical studies of the effects of SASP on serum immunoglobulins in rheumatoid arthritis and ankylosing spondylitis have shown varying results. Reductions in none, one, two or all three of the IgA, IgG, or IgM classes have been reported^{3, 49-62} in addition to SASP induced selective IgA deficiency, IgA and IgG2 deficiency, and panhypogammaglobulinaemia.⁷⁻⁹ An association between decreasing immunoglobulin concentrations and a favourable clinical response to SASP therapy has been reported^{9, 53} and corroborates our observations.

No apparent correlations between concentrations of immunoglobulins in serum and saliva or jejunal fluid were observed. This agrees with the view that the systemic and mucosal immune systems are separate. Our observations in serum do not exclude the possibility that SASP also affects immunoglobulin productivity in salivary glands and the small intestinal mucosa, although we found no evidence of such an effect. Somewhat contradictorily, Feltelius *et al* have shown, using the enzyme linked immunospot technique, that the numbers of IgA-producing peripheral blood lymphocytes were reduced after SASP treatment.⁶³ Some of these cells are proposed to originate from the gut and therefore reflect mucosal immunity;⁶⁴ however, as a considerable proportion of these circulating IgA-committed B cells originate from the bone marrow, the results of Feltelius *et al* may instead reflect the effect of SASP on systemic immunity. Indeed, an inhibitory effect of SASP on circulating IgG-committed B cells has been demonstrated using the same technique.⁶⁵

Six of our patients had increased serum IgA anti-gliadin antibody levels. The serum levels

of IgA anti-gliadin antibodies decreased significantly during SASP therapy. Our results favour the concept that this decrease simply mirrors the overall decreased production of IgA in the systemic compartment. Accordingly, SASP may regulate the production of both IgA anti-gliadin antibodies and total IgA in similar way, probably by affecting bone marrow, spleen and lymph node immunoglobulin-producing B cells. The lack of effect of SASP on jejunal IgA production further favours this concept.

Circulating IgA RF, IgA1 RF, and IgM RF all decreased during treatment with SASP. Rheumatoid factor has been observed in serum, synovial fluid, and mucosal secretions in RA patients,⁶⁶⁻⁶⁸ and production by mononuclear cells in peripheral blood, bone marrow, synovial tissues, and salivary glands has been demonstrated.^{25 69 70} IgA RF is predominantly polymeric⁷¹ and has therefore been considered to originate from mucosal tissues. However, more recent studies have shown that this RF isotype is also present and may be produced in non-mucosal compartments.^{25 70 71} Furthermore, evidence for local production of both IgA1 RF and IgA2 RF subclasses in mucosal (saliva) and in non-mucosal (synovial fluid) compartments has been put forward, and a predominance of IgA2 RF production in salivary glands compared with synovial fluid was observed.²⁵

Reduction of RF has been demonstrated after treatment with SASP and other anti-rheumatic agents, for example D-penicillamine, gold, and methotrexate.^{3 53 55 72-76} Because the changes in IgA RF and IgM RF did not correlate with the respective changes in serum immunoglobulin concentrations in the SASP treated patients (data not shown), the reductions in RF are not considered to be related to the changes in the overall production of immunoglobulins. Rather, it seems plausible that SASP directly inhibits RF production, an influence that is selective for IgA RF and IgM RF-committed B cells, as synthesis of IgG RF was not affected. A similar differential effect of antirheumatic drugs on the various RF classes has also been demonstrated for penicillamine⁷³ and gold.⁷⁴ The observed significant reduction in IgA1 RF as opposed to IgA2 RF induced by SASP treatment may further indicate a differential effect of SASP on systemic and mucosal immunity.

In summary, the results from this study indicate that SASP exerts powerful inhibitory effects on systemic IgA and IgG production, but is devoid of similar effects in mucosal tissues, judged by its lack of effect on immunoglobulin production in saliva and jejunal fluid. The effect of SASP on circulating levels of IgA anti-gliadin antibodies mirrors that on systemic immunity and favours the previously presented concept that these antibodies are not of mucosal origin.^{77 78} Finally, SASP seems selectively to inhibit production of IgA and IgM RF independently of its effect on corresponding serum immunoglobulin concentrations.

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