IgM, IgG, and IgA synthesis in vitro in persons suffering from yersinia arthritis and in patients with ankylosing spondylitis

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SUMMARY In ankylosing spondylitis (AS) and in yersinia arthritis the main findings in serum immunoglobulin (Ig) studies have been raised IgA in AS and a high anti-yersinia IgA and IgG response and the persistence of IgA class antibodies in yersinia arthritis. In order to study predisposition to high IgA response in AS and yersinia arthritis we measured the in-vitro Ig production in patients with AS and in persons who have once had yersinia arthritis, and we compared it with the Ig production in persons who have had yersiniosis but recovered without getting arthritis and with that in healthy controls. IgA secretion by peripheral blood lymphocytes stimulated by pokeweed mitogen was the same in all 4 groups, and no signs of higher IgA production in AS patients could be found. In AS patients lymphocyte activation by whole yersinia bacteria resulted in higher total IgG production than in healthy controls. The total number of plaque-forming cells in yersinia-stimulated cultures was significantly higher in persons who have had yersiniosis without arthritis than in persons who have suffered from yersinia arthritis or in persons in other groups. AS patients had low IgM production, which reverted to normal when hydrocortisone was added to the culture. The in-vitro Ig production in general did not correlate with the presence of the HLA B27 antigen but rather with the clinical history of the subjects studied.

There is evidence that gastrointestinal infection may be an important aetiological factor in ankylosing spondylitis (AS). Sacroiliitis and AS are common sequelae in persons who develop arthritis after intestinal infection with salmonella, shigella, or *Yersinia enterocolitica*.¹ Raised levels of serum IgA in patients with AS have been reported.^{2–5} Elevated serum IgA occurs predominantly during phases of active inflammatory disease.⁴ Elevated saliva secretory IgA and serum IgG have also been reported in these patients.⁵ In yersinia arthritis the IgA and IgG class anti-yersinia antibodies have been observed to persist—IgA class antibodies for even 2 to 3 years.^{6,7} Arthritic patients also have higher anti-yersinia IgA and IgG responses^{6,7} and higher concentration of anti-yersinia secretory IgA in the serum⁸ than patients who recover from yersiniosis without getting

arthritis. Both AS and yersinia arthritis are associated with the HLA B27 antigen.¹

In order to study predisposition to high IgA response in AS and yersinia arthritis we measured the in-vitro immunoglobulin (Ig) production in patients with AS and in persons who have once had yersinia arthritis. B-lymphocyte activation by yersinia was also studied. AS patients with mild subjective symptoms were chosen in order to avoid possible effects of in-vivo generated inflammatory factors on lymphocyte functions.

Materials and methods

Subjects. Forty patients were studied, divided into 4 groups. Twenty of them had suffered from *Yersinia enterocolitica* 3 infection on the average 4.5 years earlier (groups 1 and 2). Ten of these subjects had no signs of reactive arthritis (group 1, persons without arthritis = NA); 4 of them had erythema nodosum.

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All of these persons are healthy now. Ten persons had a reactive arthritis (group 2, persons with arthritis = A). Six of them are healthy now and 4 of them have arthralgias occasionally. The original diagnosis was confirmed by significantly elevated antibody titres against *Y. enterocolitica* 3. Group 3 consists of 10 patients with ankylosing spondylitis (AS). All patients met the Rome/New York diagnostic criteria for AS. The serum C-reactive protein concentration was below 10 µl/ml in 8 of them, 10 µl/ml in one, and 15 µl/ml in one patient. Two of the patients had suffered from *Y. enterocolitica* 3 infection with reactive arthritis 8 and 10 years earlier. Patients receiving anti-inflammatory analgesics were without medication for 4 days before the assay. Ten healthy blood donors served as controls (group 4). Some characteristics of the subjects are presented in Table 1.

HLA typing. The HLA typing was performed by standard 2-stage microlymphocytotoxicity tests as described by Amos *et al.*⁹

Preparation of lymphocytes. Peripheral blood lymphocytes were separated on Ficoll-Isopaque gradients (Ficoll-Paque, Pharmacia Fine Chemicals, Uppsala, Sweden) according to the method of Böyum.¹⁰ Cell viability was determined by the trypan blue exclusion method.

Induction of plaque-forming cells (PFC). 5×10^5 mononuclear cells in 1 ml of Roswell Park Memorial Institute solution (RPMI) 1640 supplemented with 50 µg/ml of gentamicin, 10% fetal calf serum (FCS, Gibco Biocult, Glasgow, Scotland), and additional 0.2% sodium bicarbonate were incubated in 12 × 75 mm round-bottomed tissue culture tubes (Falcon 2058, Falcon, Oxnard, CA). B lymphocytes were activated with formalin- and heat-treated *Staphylococcus aureus* Cowan I (SaCl, 0.0025 v/v %) bacteria, with heat-treated *Y. enterocolitica* 3 bacteria (M Y O; S. Winblad) cultured at 20°C for 48 hours (optical density of final dilution 0.025, wave length 540 nm), or with pokeweed mitogen (PWM) (2.5 µl/ml, Gibco, Grand Island, NY) in the absence or presence of hydrocortisone (HC, 10^{-5} M). The

cultures were maintained at 37°C in 15% CO₂ for 7 days.

Indirect protein-A PFC assay. The method described by Fauci *et al.*¹¹ was used. Briefly, at the end of the culture period, supernatants of the cultures were collected for measurement of Ig secretion. The cell pellet was washed once and resuspended in RPMI 1640 at an appropriate concentration. 25 µl of cells together with 850 µl agarose (Agarose Indubiose A45, Accurate Chemical and Scientific Corp., Hicksville, NY) and 50 µl of a 15% solution of sheep erythrocytes (SRBC) coupled with protein A (Pharmacia Fine Chemicals) were poured and swirled on an agarose-precoated 60 × 15 cm plastic Petri dish (Falcon 1007). The dish was kept in a CO₂ incubator at 37°C for 2 hours, when 1 ml of 1:150 dilution of polyvalent rabbit anti-human Ig (Cappel Laboratories, Cochranville, PA) was layered on the dish, which was then kept overnight in a CO₂ incubator. Thereafter the developing antiserum was removed and 1 ml of 1:40 dilution of SRBC-adsorbed guinea pig complement was added. After 1 hour's incubation the complement was removed and the plaques were enumerated. The number of PFC was calculated per 10⁶ cultured mononuclear cells (MNC).

Measurement of Ig secretion in culture supernatants. A trapping antibody ELISA method was applied for the quantitation of secreted IgA, IgG, and IgM in culture supernatants (Viljanen *et al.*, in preparation). Briefly, wells of microtitre plates (Linbro/Titertek, Flow Laboratories, Inc., Hamden, CT) were coated with rabbit anti-human IgA, IgG, and IgM (Dako-immunoglobulins a/s, Copenhagen, Denmark). Then supernatants from lymphocyte cultures were added to the wells. The bound IgA, IgG, and IgM were detected with peroxidase-conjugated rabbit anti-human IgA, IgG, and IgM (Dako-immunoglobulins a/s), respectively. The absorbance at 492 nm was measured with a Titertek Multiskan photometer (Eflab Oy, Helsinki, Finland). The amount of class-specific Ig was determined from standard

Table 1 Characteristics of the subjects studied

	Group*			
	C	NA	A	AS
Number	10	10	10	10
Men/women	5/5	5/5	4/6	7/3
Mean age in years (range)	36 (22–49)	38 (20–57)	45 (29–60)	36 (30–54)
Years from the yersinia infection: mean (range)	—	4.3 (3–6)	4.5 (3–6)	—
HLA B27 +/-	-/10	3/7	10/-	10/-

* C = healthy controls. NA = persons who have had yersiniosis without arthritis. A = persons who have had arthritis after yersinia infection. AS = patients with ankylosing spondylitis.

curves obtained from samples with known amounts of Ig (Behringwerke AG, Marburg, West Germany).

Statistical analysis. The statistical significance of the differences in the Ig production between the 4 groups was evaluated by Mann-Whitney U-test.

Results

The number of Ig producing cells was measured by a reverse PFC assay and the secretion of IgA, IgG, and IgM by measuring the amount of these immunoglobulins in the culture supernatant. There was a tendency towards high PFC numbers in persons who had had yersiniosis without arthritis and towards low PFC numbers in patients with AS. In unstimulated cultures there were no significant differences in the PFC numbers between the 4 groups (Fig. 1). In yersinia-stimulated cultures persons without arthritis had significantly higher PFC numbers than subjects in other groups (NA>C, $p<0.001$; NA>A, $p<0.025$; NA>AS, $p<0.05$) (Fig. 1). In SaCI-stimulated cultures persons without arthritis again had significantly higher PFC numbers than subjects in other groups (NA>C, $p<0.001$; NA>A, $p<0.05$; NA>AS, $p<0.05$) (Table 2). The number of PFC in the PWM-stimulated cultures of AS patients was significantly lower than that in persons with or without arthritis (AS<NA, $p<0.05$; AS<A, $p<0.05$) (Fig. 1). When the cells were cultured together with PWM and hydrocortisone this difference in the PFC number could no more be detected (data not shown).

When the Ig isotype secretion was measured, the main difference between the 4 groups was found in the IgM production. In unstimulated cultures the

Table 2 Total Ig producing cells (PFC)/ 10^6 cultured mononuclear cells

Group*		Unstimulated	SaCI-stimulated
C	Geometric mean	500	2100†
	Range	100–3300	1100–4800
NA	Geometric mean	1200	5000
	Range	400–11 000	3200–9000
A	Geometric mean	800	2400†
	Range	100–4500	900–18 000
AS	Geometric mean	500	2700†
	Range	300–1400	700–7500

* C = healthy controls. NA = persons who have had yersiniosis without arthritis. A = persons who have had arthritis after yersinia infection. AS = patients with ankylosing spondylitis.

† NA>C, $p<0.001$; NA>A, $p<0.025$; NA>AS, $p<0.05$.

IgM production was higher in persons who had had yersinia infection than in AS patients or in controls. The difference was significant in comparison with AS patients (NA>AS, $p<0.01$; A>AS, $p<0.025$) (Fig. 2). In yersinia-stimulated cultures persons without arthritis had a higher IgM production than subjects in other groups. The difference was significant in comparison with controls and with AS patients (NA>C, $p<0.001$; NA>AS, $p<0.001$) (Table 3). The production of IgM in PWM-stimulated cultures from AS patients was significantly lower than that for subjects in other groups (AS<C, $p<0.05$; AS<NA, $p<0.01$; AS<A, $p<0.025$) (Fig. 2). When the cells were stimulated with PWM in the presence of hydrocortisone the increase in IgM production, as compared with PWM alone, was most clear in AS patients (PWM+HC>PWM, AS $p<0.0025$; NA $p<0.005$;

Fig. 1 Total Ig producing cells (PFC)/ 10^6 cultured mononuclear cells (MNC) in unstimulated cultures and in yersinia- and PWM-stimulated cultures in healthy controls (C), in persons who have had yersiniosis without arthritis (NA), in persons who have had arthritis after yersinia infection (A), and in patients with ankylosing spondylitis (AS). Significant differences were found only in yersinia-stimulated cultures: NA>C, $p<0.001$; NA>A, $p<0.025$; NA>AS, $p<0.05$. (●) = HLA B27 negative subject and (■) = HLA B27 positive subject.

Fig. 2 The amount of IgM (ng/ml) secreted into the culture supernatant in unstimulated cultures and in cultures stimulated with PWM or with PWM + hydrocortisone (HC) in healthy controls (C), in persons who have had yersiniosis without arthritis (NA), in persons who have had arthritis after yersinia infection (A), and in patients with ankylosing spondylitis (AS). Significant differences were found in unstimulated cultures: NA>AS, $p<0.01$; A>AS, $p<0.025$, and in PWM-stimulated cultures: C>AS, $p<0.05$; NA>AS, $p<0.01$; A>AS, $p<0.025$. (●) = HLA B27 negative subject and (■) = HLA B27 positive subject.

Table 3 Amount of immunoglobulins (ng/ml) secreted into the culture supernatant

Groups*		Unstimulated			Yersinia-stimulated		PWM-stimulated	
		IgM	IgG	IgA	IgM	IgG	IgG	IgA
C	Geometric mean	32	229	40	63†	290	629	111
	Range	10-190	150-410	10-230	10-360	150-770	140-5780	10-1920
NA	Geometric mean	55‡	217	38§	327	483''	761	250
	Range	20-100	140-630	10-240	120-1200	170-950	530-1150	60-980
A	Geometric mean	78‡	244	27§	151	478''	663	154
	Range	30-520	120-450	10-90	30-410	310-1020	220-1670	60-620
AS	Geometric mean	34	349	75	95†	513''	589	129
	Range	20-60	120-810	30-230	10-250	170-1150	140-1950	50-450

* C = healthy controls. NA = persons who have had yersiniosis without arthritis. A = persons who have had arthritis after yersinia infection. AS = patients with ankylosing spondylitis.

† NA>C, $p<0.001$; NA>AS, $p<0.001$.

‡ NA>AS, $p<0.01$; A>AS, $p<0.025$.

§ AS>NA, $p<0.05$; AS>A, $p<0.05$.

'' NA>C, $p<0.05$; A>C, $p<0.05$; AS>C, $p<0.05$.

A $p<0.0125$; C $p<0.05$, paired t test), and there were no more differences in the IgM production between the 4 groups (Fig. 2).

The only difference between the 4 groups in the IgG production was observed in yersinia-stimulated cultures: the IgG production was significantly higher in subjects of the 3 other groups in comparison with controls (NA>C, $p<0.05$; A>C, $p<0.05$; AS>C, $p<0.05$) (Table 3).

The production of IgA in PWM-stimulated cultures was on the same level in all groups (Table 3). Yersinia stimulated IgA production only weakly: in yersinia-stimulated cultures the mean stimulation index (SI = IgA in stimulated culture/IgA in unstimulated culture) was 1.5 and the range was 0.2-5. In unstimulated cultures AS patients had significantly higher IgA production when compared with persons

with or without arthritis (AS>NA, $p<0.05$; AS>A, $p<0.05$) (Table 3).

All subjects studied were typed for HLA B27 antigen. The differences in the Ig production were not correlated with the presence of this antigen but rather with the clinical history of the subject.

Discussion

The purpose of this study was to compare the in-vitro Ig production in persons who have once had yersinia arthritis and in patients with AS with that in persons who have had yersiniosis without arthritis and with healthy controls without any history of yersiniosis. The main interest was focused primarily on the IgA production because of reports of elevated serum IgA

in active AS²⁻⁵ and higher anti-yersinia IgA in yersinia arthritis.^{6,7} AS patients showed slightly higher IgA production in unstimulated cultures. However, yersinia did not stimulate IgA production to any significant extent, and in PWM-stimulated cultures there were no differences in the IgA production. This indicates that AS patients and persons who have suffered from yersinia arthritis have no predisposition to a high IgA response in vitro. On the other hand peripheral blood lymphocytes (PBL) may not be the right cells when studying the IgA production in these diseases, since the in vitro response with PWM-stimulated IgA secretion of PBL is more than 90% of the subclass IgA₁ and less than 10% of the subclass IgA₂ (mainly found in vivo in secretions).¹² For the IgA₂ secretion probably microenvironmental factors, present in the secretory tissues, are needed. In this respect our results may reflect the IgA₁ secretion and do not rule out the possibility of an altered immune stimulation or response in the gut in AS and yersinia arthritis.

In the earlier studies we observed that patients with yersinia arthritis have a weaker lymphocyte transformation response to whole yersinia bacteria than patients who recover from yersiniosis without arthritis.¹³ This depressed response in arthritic patients can be found even years after the acute infection (Vuento *et al.*, unpublished observations). Our present finding of depressed B-lymphocyte activation by yersinia in persons suffered from yersinia arthritis is in accordance with those observed by the lymphocyte transformation test. Depression in both Ig production and blast transformation, notwithstanding that they are distinct phenomena, may indicate an altered immunoregulation in yersinia arthritis. On the other hand elevated yersinia-stimulated IgG production in AS patients suggests that enterobacteria have a role in the causation of AS.

The most striking finding was the low IgM production in vitro in PWM-stimulated cultures from AS patients. When the cells were cultured together with PWM and HC, the IgM production in AS patients normalised. The IgM production, like the IgA and IgG production, showed no correlation with the presence of HLA B27 antigen but rather with the clinical history of the patient. In AS patients serum IgM has been reported to be normal,^{4,5} but the specific anti-*klebsiella* IgM has been found to be low,¹⁴ as well as the specific anti-yersinia IgM in yersinia arthritis.⁷ In other connective tissue diseases, such as SLE and rheumatoid arthritis (RA), despite high levels of immunoglobulins in the serum, decreased production of Ig (especially IgM but also IgG) in vitro has been found.¹⁵⁻¹⁷ The significance of this impaired IgM synthesis by PBL in vitro is not clear. In diseases with a definite triggering agent, like yersinia arthritis, a

low specific IgM response might reflect a weak first-phase immune defence. In chronic diseases of more or less unknown origin there may be a polyclonal B-lymphocyte activation in vivo.¹⁸ As a consequence of increased suppression, PBL may show in vitro a low IgM production. The presence of suppressor lymphocytes is suggested by our finding that HC, which inhibits spontaneous suppressor cells,¹⁹ normalises the suppressed PWM-stimulated production of IgM and the low number of PFC in AS patients.

In conclusion, persons who have suffered from yersinia arthritis have depressed yersinia-induced in-vitro total Ig production in comparison with those who have recovered from yersiniosis without getting arthritis, suggesting an altered immunoregulation in yersinia arthritis. When PBL are studied, AS patients and persons who have suffered from yersinia arthritis show no predisposition to higher total IgA response than normal controls, whereas in AS the PWM-stimulated IgM response is depressed. The role of suppressor cells in the regulation of IgM synthesis in AS remains to be discovered.

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Book review

Hypermobility of Joints. By P. Beighton, R. Grahame, H. Bird. Pp. 178. £29.00. Springer-Verlag: London. 1983.

It is unusual to find that one has read a book from cover to cover, has learned a great deal, has benefited from the authors' success in bringing together the many aspects of the subject, and has enjoyed the clarity and brevity of presentation, while finding little if anything which merits criticism. This volume has satisfied all these points, and the authors are to be congratulated on the undoubted success of their work in producing this comprehensive account of the hypermobility syndrome. As they point out, the hypermobile individual acting as a contortionist has been known

since ancient times. However, the scientific and medical implications of hypermobility have been realised and investigated only in the last 50 years and particularly in the last two decades. The present understanding of collagen abnormalities and their association with other conditions are well described. From a practical point of view diagnosis, prevalence, and clinical features are comprehensively and well covered. The 30 pages of clinical accounts of patients with the hypermobility syndrome are most valuable.

This monograph is an essential volume for all rheumatology and orthopaedic libraries and also deserves a place in medical libraries in general in view of the frequency of the syndrome, the overlap with other conditions, and its many and varied manifestations.

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