Increased levels of serum IgA as IgA₁ monomers in ankylosing spondylitis

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Abstract
The various subsets of serum IgA were determined in 43 patients with ankylosing spondylitis to investigate the putative mucosal origin of increased IgA concentrations in this disease. Total IgA was shown to be increased and weakly correlated with the erythrocyte sedimentation rate (ESR). In contrast, although the mean concentration (but not the median) of secretory IgA (SIgA) was slightly increased, no correlation was found with total IgA nor the ESR. Moreover, molecular sieving of nine serum samples selected for their high concentrations of total IgA, and absorption with insoluble jacalin showed these immunoglobulins to be essentially monomers of the IgA₁ subclass. These results are consistent with a non-secretory origin of the increase of serum IgA, which must be ascribed to the central immune system.

Several workers have reported abnormally high concentrations of serum IgA in patients with ankylosing spondylitis and it has been proposed that this increase is related to a stimulation of the mucosal compartment.[1-10] IgA is the major isotype produced by the mucosal associated lymphoid tissue and the secretory immune system is assumed to play an important part in the pathophysiology of ankylosing spondylitis. Certain components of gut microorganisms were found to be cross reactive with the ankylosing spondylitis associated HLA-B27 antigen (reviewed by Yu *et al*[11]) and intestinal lesions are common in this disease.[12]

Serum IgA are essentially 160 kilodalton monomers (mIgA), produced by the central immune system. The polymeric forms (pIgA) are mainly covalent dimers (330 kilodaltons), representing about 13% of serum IgA, and are produced by the central immune system and by the mucosal associated lymphoid tissue. A minor subset (11·5 μg/ml) of polymers (400 kilodaltons) is similar to secretory IgA (SIgA). The IgA₁ subclass represents about 90% of serum IgA, whereas the IgA₁/IgA₂ ratio varies from 0·7 to 2 in mucosal associated lymphoid tissue and secretions.[13] A putative relation between IgA increases and the mucosal associated lymphoid tissue led us to analyse the different forms of IgA in the serum of patients with ankylosing spondylitis.

We show here that the high concentrations of serum IgA are essentially due to monomers of the IgA₁ subclass, known to almost exclusively originate from the central immune system.

Patients and methods
A group of patients with ankylosing spondylitis, 38 men and five women, aged from 19 to 64 years (median 33 years) was investigated (table 1). None of these subjects had associated psoriasis, enteritis, or liver diseases. A pool of 250 serum samples from normal blood donors served as a control group.

Total IgA was measured by single radial immunodiffusion using a sheep antihuman α chain antiserum, prepared in our laboratory. The assay was carried out in duplicate and at two different dilutions, and compared with three dilutions of the pool of normal serum samples. The concentration of SIgA—that is, pIgA containing covalently bound secretory component—was measured by a capture enzyme linked immunosorbent assay (ELISA) according to the method of Kvale and Brandtzaeg,[14] using plates coated with our sheep antihuman secretory component antibodies.[14 15] The captured serum SIgA was visualised with peroxidase labelled rabbit antihuman α chain antibodies. The assay was carried out in duplicate at three dilutions and the results compared with an internal control slope obtained from five duplicate dilutions of the reference normal serum pool.

The degree of polymerisation of IgA was investigated in nine serum samples, selected for their high IgA contents, by gel permeation chromatography using a series of three 1·5×100 cm columns of S300 Sephacryl (Pharmacia, Uppsala, Sweden) equilibrated in phosphate buffered saline containing 2 M NaCl. Under these conditions, non-covalent IgA aggregates—that is, 'false' polymers—dissociate and are eluted with the mIgA fraction (personal observation, unpublished), whereas 'true' (covalent) pIgA remain unchanged and are eluted before mIgA. Effluents were collected as 3 ml fractions and the concentration of IgA measured by capture ELISA with sheep antihuman α chain antibody coated plates and the labelled antihuman α chain antibodies.

Jacalin affinity[16] was used to quantify the IgA₁ subclass molecules. This lectin was extracted and purified from jackfruit seeds of Vietnamese origin and treated with CNBr on Sepharose 4B beads (Pharmacia). The Sephacryl fractions corresponding to pIgA or mIgA were pooled and incubated with this sorbent. The amount of IgA₁ was estimated by difference between the concentrations of total IgA before and after absorption.

STATISTICAL ANALYSIS
The mean concentration was compared with the

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value of the serum pool using Student's t test. In addition, the number of patients with concentrations higher than the control value was compared with that of patients with concentrations less than this value using the χ^2 test. Correlation coefficients were investigated by linear regression analysis. The probabilities were drawn by intrapolation of the statistical tables of Fisher and Yates.

Results

The mean (SD) concentration of serum IgA for the patients with ankylosing spondylitis was 3·10 (1·3) mg/ml and was significantly higher ($p<10^{-6}$) than the normal control concentration of 2·2 mg/ml (table 1). Similarly, the median

value was 3 mg/ml and the proportion of serum samples with concentrations greater than (34/43), or less than (9/43) the control value was significantly different ($p<0.0003$) from 50%. A weak correlation ($r=0.337$, $p<0.03$) was observed with the ESR, whereas the concentration of serum IgA varied independently of the disease duration ($r=-0.028$).

The mean (SD) concentration of serum SIgA (18·3 (17·2) µg/ml) was slightly increased ($p<0.02$), whereas the median (12 µg/ml) was similar to the value of the control serum pool (11·5 µg/ml). This discrepancy between mean and median values is due to a bimodal distribution, 6/43 patients (patients 3, 16, 18, 20, 21, and 23) showing high levels. No individual correlation was found between SIgA and total serum IgA ($r=-0.012$), ESR ($r=0.141$), or disease duration ($r=-0.09$).

Analysis of the molecular mass by gel permeation chromatography showed the proportion of mIgA and pIgA to be in the normal range in all the selected serum samples, and thus these subsets were accordingly increased (table 2). Subclass analysis by absorption with insoluble jacalin showed pIgA and mIgA pools to contain a high proportion of IgA_1, compared with the normal serum pool (table 2).

Discussion

Addressing the question of a relation between the increased concentration of serum IgA and an eventual stimulation of the mucosal associated lymphoid tissue in patients with ankylosing spondylitis, we investigated the molecular status of this increase. We confirm that serum concentrations of IgA are often increased in these patients, but show this increase to be due essentially to mIgA—that is, to molecules mainly produced by the central immune system.[17] In addition, the proportion of IgA_1 is in the normal range in serum mIgA and pIgA, confirming their central origin.

In agreement with numerous reports,[1-10] we found the serum concentration of IgA to be slightly, but often, increased in our patients, although nine had a concentration less than that of the normal control serum pool. The positive correlation between serum IgA and the ESR was weak ($p<0.03$), explaining why it was not constantly observed in previous studies.

The method used here to quantify SIgA detected this molecule alone, irrespective of other pIgA, as capture occurred through a highly specific antibody to secretory component.[14 15] The SIgA concentration in our pool was considered to be the true value of the whole normal population and thus was used as an internal standard for each measurement. This reference value is between the values of 10–12 µg/ml given by various other groups.[15 18-20] The slight discrepancy between the mean and median comparisons, due to a bimodal distribution of the patients, needs to be confirmed with other independent series of patients. Nevertheless, none of the SIgA results seen can explain even a weak increase of total IgA. Furthermore, in contrast with data suggesting a slight increase of serum SIgA during the active

Table 1 Patient characteristics

Patient No	Disease duration (years)	ESR* (mm/h)	Serum IgA (mg/ml)†	Serum SIgA (µg/ml)‡
1	6	42	2	12
2	1	50	3·2	20
3	11	53	1·9	48
4	4	5	3	8
5	1	40	3·9	20
6	17	25	4·2	13
7	10	16	2·6	25
8	0·5	5	1	12
9	4	50	4·8	14·5
10	0·5	60	3·2	15
11§	39	30	2·4	10
12	0·2	34	3·3	12
13	16	12	3	20
14	1	10	1·6	12
15	16	5	3·6	29
16	4	60	3·9	60
17	5	2	0·9	16
18	5	12	4·1	58
19	6	52	2	23
20	4	29	2·7	48
21	14	45	3·2	65
22	2	22	3·3	15
23§	6	67	2·4	67
24	4	62	7	10
25§	3	12	6	11
26	1	15	1·5	5
27	10	10	2·7	12
28	16	15	2·9	5
29	3	2	1·6	6
30	1	40	2·8	11
31	14	38	3·2	10
32§	16	10	2·9	11
33	3	53	6·4	7
34	20	68	2·4	7·7
35	12	48	3·9	8·1
36	8	35	2·8	8·7
37§	0·5	6	1·5	7·4
38	15	23	4	7·3
39	3	100	4·5	10
40	14	45	3·1	2·3
41	10	40	2·7	9·2
42	1	87	3	5·7
43	2	28	2·3	9

*(ESR) erythrocyte sedimentation rate.
†Median 3; mean (SD) 3·1 (1·3); normal pool 2·2 mg/ml.
‡Median 12; mean (SD) 18·3 (17·2); normal pool 11·5 mg/ml.
§Female patients.

Table 2 Proportions of polymers and IgA_1 in selected serum samples

Patient No*	IgA (mg/ml)	pIgA (%)	IgA_1 (%)	
			pIgA pool	mIgA pool
24	7	11·6	95·7	96
33	6·4	9·3	96·4	99·1
25	6	13·1	97	96·6
9	4·8	14·4	95·4	95·9
39	4·5	6	96	92·9
6	4·2	9	95·3	97
18	4·1	15·6	96·6	97
35	3·9	12	97·6	96·4
16	3·9	6	96·6	97
Normal serum pool	2·2	12·5	89·5	93·5

*Selected for their high serum IgA concentrations (decreasing order).

stages of the disease,[21] our results showed no correlation with the ESR or with total IgA. Although SIgA cannot be involved in the increased concentration of serum IgA in our patients, this study is not at variance with a possible part played by the mucosal associated lymphoid tissue in the aetiopathology of ankylosing spondylitis. Indeed, the concentration of serum SIgA has been shown to be normal in gut inflammation,[20] [22] and we have proposed a central origin for these secretory-like molecules.[23]

Size analysis of our selected serum samples showed a normal proportion of pIgA in ankylosing spondylitis, leading to the conclusion that mIgA mainly accounts for the increase in IgA in these patients. Hence this IgA disorder appears to involve molecules produced by the central immune system, without a direct relation with the mucosal associated lymphoid tissue. This evidence is also supported by the high proportion of IgA_1 in these serum samples, compared with the classical range of 39–63% in normal gut secretions.[24] Moreover, similar high proportions of this subclass were found in pIgA and mIgA (table 2).

Increased mIgA in serum samples can also occur in Berger's disease, where it is similarly ascribed to an overproduction of the central immune system.[25] [26] Interestingly, this disease has often been observed in patients with ankylosing spondylitis (reviewed by Lai *et al.*[27]), and common mechanisms could be shared by these two disorders. The reason why mIgA is increased remains unexplained. Nevertheless polysaccharides, namely bacterial components, have been shown to induce preferably IgA antibodies, and studies have emphasised the importance of interleukin 4, 5, and 6 in the selective production of IgA.[28] Prolonged immunisation against putative digestive bacteria causing ankylosing spondylitis or the release of interleukins out of inflamed joints or mucosae, or both, might be alternative explanations for the increase of IgA in patients with ankylosing spondylitis.

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