EXTENDED REPORTS

Serum immunoglobulins and the risk of rheumatoid arthritis

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Abstract

Objective—Rheumatoid arthritis (RA) is associated with several autoantibodies that can precede the clinical disease. The immunoglobulin concentrations in serum samples before illness were studied to learn more about the immunological process before RA.

Methods—A case-control study was nested within a Finnish cohort of 19 072 adults who had neither arthritis nor a history of it at the baseline examination during 1973–1977. By late 1989, 124 had developed RA, of which 89 were positive for rheumatoid factor (RF). Three controls per each incident case were individually matched for sex, age, and municipality. The concentrations of IgG, IgA, and IgM were measured from stored serum samples.

Results—Serum IgG before illness was found to be directly proportional to the risk of RF positive RA, and a non-linear association was present between serum IgA and the risk of RF positive RA. These associations were constant between men and women and other subgroups of the study population and not confounded by serum orosomucoid concentration, level of education, smoking, alcohol intake or body mass index. As adjusted for these factors, the odds ratios (95% confidence intervals) of RF positive RA in the lowest, mid, and highest tertiles of IgG distribution were 1.00, 1.55 (0.81, 2.97), and 2.22 (1.16, 4.26), and in the tertiles of IgA 1.00, 2.23 (1.14, 4.36), and 1.78 (0.89, 3.57), respectively. The associations persisted throughout the entire observation period but were most distinct when the period to the onset of clinical RA was ≥ 10 years. IgM carried no predictive significance. None of the serum immunoglobulins predicted the development of RF negative RA.

Conclusions—Increased IgG levels may reflect some, at present unknown process in the early events leading to the development of RA, typically occurring ≥ 10 years before the onset of clinical disease.

Patients with rheumatoid arthritis (RA) have higher immunoglobulin IgG, IgA, and IgM concentrations than appropriate controls.¹⁻³ After treatment with disease modifying anti-rheumatic drugs the values tend to decline⁴⁻⁶; thus, the increased immunoglobulin concentrations obviously reflect in one way or another the underlying immunological process of RA.

RA is associated with several autoantibodies specific enough to serve as diagnostic and prognostic markers of the disease.⁷⁻¹¹ Of these, rheumatoid factor (RF) and the two closely related antibodies, antikeratin antibody (AKA) and antiperinuclear factor, have been shown to precede the onset of clinical RA.⁷⁻⁹ The prevalence of positive reactions for these antibodies in specimens taken before the onset of illness is dependent on the period between the taking of specimens and onset of disease; when the period is short, the prevalence is nearly the same as in the established disease. It is possible that there are many other recognised or as yet unrecognised autoantibody systems that herald the onset of RA.

One specific antibody usually constitutes only a tiny fraction of the immunoglobulin pool, yet the total contribution of autoantibodies or other antibodies involved in the pre-rheumatoid immunological process might be substantial. To learn more of this process, we investigated the association between serum immunoglobulin concentrations and risk of RA in initially healthy subjects.

Methods

STUDY POPULATION

During 1973–1977, the Mobile Clinic Unit of the Social Insurance Institution undertook multiphasic health examinations in 12 municipalities in four regions of Finland, in which the main emphasis was on the risk factors for cardiovascular disorders. In each of the four geographical regions, all inhabitants or a random sample of inhabitants of one rural municipality and one urban or semiurban municipality as well as the employees of one factory were invited to attend the examination. A total of 19 518 subjects ≥ 20 years of age (83% of those invited) participated in the

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examinations. The mean age of the subjects was 45 (age range 20–98).

BASILINE EXAMINATION

A questionnaire with items concerning socioeconomic background, medical history, smoking, alcohol consumption and physical activity was sent to the subjects together with the invitation to the medical checkup, for completion before the examination. The answers to this self-completed questionnaire were checked and amended where necessary, by a specially trained nurse at the examination. The overall consumption of alcohol was calculated by multiplying the average intakes of beer, wine, and strong beverages during the preceding month by the average alcohol content of each beverage and this overall consumption was expressed both in grams and in units (4 cl including 13.2 grams of ethanol) per month. Education was used as a measure for socioeconomic status and was classified as follows: low education, including primary school (8 years) or less; intermediate education, including junior high school, comprehensive school, vocational school, technical school, or high school (9–13 years); and high education, including studies or degrees taken at institutes or universities (over 13 years).

The history of diseases was obtained with two questions: 'Have you any defect, injury, or chronic illness that reduces your general working capacity?' and 'Have you any other defect, injury or illness?' and, if yes: 'Define or describe these impairments or illnesses.' The diseases were coded by specially trained research assistants after the field survey. Of all the participants 446 (2.3%) had a history of RA or other inflammatory arthritis, or had been entitled to specially reimbursed anti-rheumatic medication or, if entitled to it later, had the onset of joint symptoms before the baseline examination.

IDENTIFICATION OF INCIDENT CASES AND MATCHED CONTROLS

The population at risk consisted of participants who had no previous history of arthritis or other rheumatoid disease according to the Social Insurance Institution's population register or to a questionnaire administered at the baseline examination. The morbidity and mortality of all examinees were followed up continuously. Participants in the survey who later developed chronic arthritis were identified by linking the survey data with the Social Insurance Institution's population register up to the end of 1989, using the unique identification code assigned to each Finnish citizen. In Finland reimbursed drugs are provided for certain chronic diseases, including chronic inflammatory rheumatic diseases. Eligibility requires a comprehensive medical certificate written by the attending physician. These written documents were carefully reviewed to identify incident cases of RA on the basis of the following criteria: (1) The patient had been granted the right to specially reimbursed medication because of physician's diagnosis of clinical RA; (2) there was no

history of rheumatoid disease at the health examination; and (3) according to the doctor's certificate, the onset of disease was after the health examination. Information on the RF status was obtained from the certificates. Testing had been performed in several different laboratories.

The sickness insurance offices keep patient documents for only three years. In addition, there were a few cases in which the documents were lost or the information was incomplete, for instance with respect to RF status. In some of the above cases, the missing data were obtained from other sources, such as hospital records. Sufficient information concerning diagnosis, onset of disease, and RF status was obtained on 124 cases. Three controls per case were selected by individual matching, using sex, municipality, and age as matching factors. Matching for municipality also controlled for both the time of the baseline examination and for the duration of storage of serum samples.

LABORATORY METHODS

Serum samples were drawn and kept frozen at -20°C until thawed in 1994. Serum immunoglobulin IgG, IgA, and IgM and orosomucoid concentrations were quantified by standard immunoturbidimetry, using a Kone Specific automated clinical chemistry analyser (Kone Instruments, Espoo, Finland). The standards had been calibrated with the IFCC/BCR CRM 470-standard and they were from Labquality Inc (Helsinki, Finland). Antisera were from Dako A/S (Glostrup, Denmark). The coefficient of interassay variation for the analytical reproducibility ranged from 4.4% (orosomucoid) to 5.7% (IgG).

The sensitised sheep cell agglutination (Waller-Rose) test for RF was performed on microtitre plates with U shaped wells.¹² Sheep red cells were sensitised with one third of the minimum agglutination dose. The plates were stored overnight at 4°C in a refrigerator, after which the readings were taken on the basis of sedimentation patterns. Titres ≥ 32 were regarded as positive.

AKA were assayed by the indirect immunofluorescence technique using unfixed sections of the mid third of rat oesophagus as the antigen source.¹³ The serum samples were diluted 1:10 and 1:50. Fluorescein isothiocyanate labelled sheep antihuman IgG (National Bacteriological Laboratory, Stockholm, Sweden) was used to detect bound antibodies. Only laminar staining of the stratum corneum was interpreted as a positive finding.

STATISTICAL ANALYSIS

Associations between serum immunoglobulin concentrations and other personal characteristics were assessed with the general linear model.¹⁴ The significance of differences in mean concentrations of serum immunoglobulins between RA cases and their matched controls was tested using the paired *t* test. The conditional logistic model¹⁵ was used to estimate the associations between serum immunoglobulin concentrations and the risk of RA. Potential confounding factors were also

Table 1 Baseline characteristics of incident cases with RF positive, RF negative, and total RA and their matched controls

Characteristic	RF positive RA				RF negative RA				Total RA			
	Number of sets	Cases	Controls	p Value	Number of sets	Cases	Controls	p Value	Number of sets	Cases	Controls	p Value
IgG (g/l)	88	15.2 (4.2)	14.6 (3.3)	0.21	34	15.3 (4.3)	15.1 (3.0)	0.79	122	15.3 (4.2)	14.7 (3.2)	0.22
IgA (g/l)	88	2.7 (1.1)	2.7 (0.8)	0.82	34	2.4 (1.5)	2.5 (0.8)	0.73	122	2.6 (1.2)	2.6 (0.8)	0.97
IgM (g/l)	88	1.8 (0.8)	1.9 (0.8)	0.27	34	1.8 (0.8)	1.9 (0.5)	0.22	122	1.8 (0.8)	1.9 (0.8)	0.13
Orosomucoid (mg/l)	89	720 (190)	726 (144)	0.79	34	648 (197)	701 (116)	0.14	123	700 (194)	719 (137)	0.31
Body mass index (kg/m ²)	89	25.8 (4.1)	25.4 (2.9)	0.40	35	25.3 (4.5)	26.3 (2.8)	0.20	124	25.6 (4.2)	25.6 (2.9)	0.99
Smoking	89				35				124			
Never (%)		51.7	60.9	0.05		71.4	65.7	0.41		57.3	62.2	0.20
Stopped (%)		18.0	14.6	0.45		8.6	13.3	0.28		15.3	14.2	0.75
Currently (%)		30.3	24.5	0.24		20.0	21.0	0.90		27.4	23.5	0.35
Alcohol intake	88				35				123			
Not at all (%)		44.3	43.9	0.95		60.0	53.3	0.48		48.8	46.6	0.65
≤ 99 g (7.5 units)/month		20.5	19.5	0.84		8.6	20.0	0.06		17.1	19.6	0.50
≥ 100 g (7.6 units)/month		35.2	36.6	0.80		31.4	26.7	0.58		34.1	33.7	0.93
Education	89				35				124			
Low (%)		71.9	71.5	0.94		77.1	72.4	0.57		73.4	71.8	0.70
Intermediate (%)		21.3	23.6	0.63		14.3	21.0	0.38		19.4	22.8	0.38
High (%)		6.7	4.9	0.55		8.6	6.7	0.74		7.3	5.4	0.50

Data shown as mean (SD).

entered into the model. The relative odds with 95% confidence intervals were computed for tertiles of serum IgG, IgA, and IgM. Linear trends between serum immunoglobulin concentrations and RA risk were tested with the likelihood ratio test based on the model and expressed as exact p values.

Results

The sensitised sheep cell agglutination (Waller-Rose) test for RF was positive in seven of 368 controls (2%), in 27 of 89 (30%) preillness specimens from subjects who developed RF positive RA, and in none of 35 subjects who developed RF negative RA. Of the preillness specimens from RF positive cases, nine of 18 (50%) were positive when the interval between taking of the specimen and the onset of disease was less than five years, nine of 31 (29%) specimens were positive when the interval was five to nine years, and nine of 40 (22%) specimens were positive when the interval was 10 years or more.

The test for AKA was positive in three of 368 controls (1%), in 13 of 89 (15%) preillness

specimens from subjects who developed RF positive RA, and in two of 35 (6%) preillness specimens from subjects who developed RF negative RA. In preillness specimens, the test for AKA was positive in 25% when the interval between taking of the specimen and the onset of disease was less than five years, in 10% when the interval was five to nine years, and in 8% when the interval was 10 years or more.

The mean concentration of serum IgG was 0.6 g/l higher among future incident cases of RF positive RA than their controls, but the difference was not statistically significant. There were no differences in the mean concentrations of serum IgA or IgM between the cases with either RF positive or RF negative RA and their controls (table 1).

Several factors were associated with serum immunoglobulin concentrations and could thus act as potential confounders or effect modifiers of the association between immunoglobulins and RA risk (table 2). As adjusted for sex and age, a positive correlation was present between serum orosomucoid and IgA

Table 2 Partial correlations between serum immunoglobulins and other baseline characteristics and category specific means of serum immunoglobulins adjusted for age and sex among the 363 controls

	IgG			IgA			IgM		
	Partial correlation ratio	Adjusted mean	p Value	Partial correlation ratio	Adjusted mean	p Value	Partial correlation ratio	Adjusted mean	p Value
Sex*	0.10		0.05	0.15		0.006	-0.33		<0.001
Men		14.1			2.9			1.3	
Women		15.0			2.5			2.2	
Age†	0.01		0.78	0.14		0.008	-0.13		0.02
Orosomucoid	0.00		0.98	0.12		0.04	-0.01		0.90
Body mass index	-0.02		0.67	0.02		0.68	-0.12		0.03
Smoking	0.17		0.005	0.10		0.13	0.11		0.11
Never		14.8			2.7			2.0	
Stopped		16.3			2.7			2.1	
Currently		13.8			2.4			1.7	
Alcohol intake	0.11		0.13	0.09		0.25	0.18		0.004
Not at all		15.2			2.6			1.8	
≤ 99 g (7.5 units)/month		14.9			2.9			2.3	
≥ 100 g (7.6 units)/month		14.0			2.6			1.9	
Education	0.06		0.52	0.09		0.22	0.11		0.12
Low		14.6			2.6			1.8	
Intermediate		15.2			2.7			2.1	
High		14.8			3.1			2.0	

* Adjusted for age only, † adjusted for sex only.

Table 3 Relative risks (odds ratios with 95% confidence intervals, CI) of RF positive RA, RF negative RA, and total RA among tertiles* of serum IgG, IgA, and IgM concentrations

Tertiles	RF positive RA				RF negative RA				Total RA			
	Number of cases	Number of controls	Odds ratio	95% CI	Number of cases	Number of controls	Odds ratio	95% CI	Number of cases	Number of controls	Odds ratio	95% CI
IgG												
Lowest	22	941	1.00		9	28	1.00		31	119	1.00	
Middle	31	87	1.45	0.77–2.71	10	33	0.96	0.35–2.68	41	120	1.30	0.76–2.22
Highest	35	79	1.88	1.01–3.52	15	39	0.21	0.43–3.37	50	118	1.68	0.99–2.86
p for trend			0.01				0.69				0.05	
IgA												
Lowest	18	82	1.00		15	38	1.00		33	120	1.00	
Middle	36	84	1.93	1.02–3.66	12	35	0.84	0.34–2.06	48	119	1.48	0.89–2.47
Highest	34	91	1.67	0.86–3.24	7	27	0.62	0.20–1.94	41	118	1.28	0.74–2.22
p for trend			0.14				0.41				0.37	
IgM												
Lowest	29	87	1.00		14	32	1.00		43	119	1.00	
Middle	31	85	1.06	0.59–1.90	9	35	0.53	0.18–1.55	40	120	0.90	0.54–1.49
Highest	28	85	0.98	0.52–1.83	11	33	0.69	0.26–1.84	39	118	0.89	0.53–1.51
p for trend			0.95				0.48				0.67	

* The tertiles are based on the distribution of concentrations (g/l) among controls. Tertile divisions are, for IgG < 12.21, 12.22–15.00, > 15.00 among men and < 12.97, 12.97–15.33, > 15.33 among women; for IgA < 2.12, 2.12–3.10, > 3.10 among men and < 1.87, 1.87–2.76, > 2.76 among women; and for IgM < 1.06, 1.06–1.52, > 1.52 among men and < 1.57, 1.57–2.41, > 2.41 among women.

and an inverse correlation between body mass index and IgM. Serum IgG varied significantly among the smoking categories ($p = 0.005$), and was highest in ex-smokers and lowest in current smokers.

Serum IgG was found to be directly proportional to the risks of RF positive RA (table 3). An association was also present between IgA and the risk of RF positive RA; the odds ratio was significantly increased in the midtertile compared with the lowest tertile of IgA distribution. Serum IgM carried no predictive significance. None of the serum immunoglobulins predicted the development of RF negative RA. Because the controls for RF positive and RF negative RA cases were unevenly distributed between the tertiles of IgG and IgA (table 3) the tertile divisions were also determined separately for these subgroups.

Using these group-specific divisions, the odds ratios remained essentially the same as those presented in table 3.

The association between IgG and risk of RF positive RA was not confounded by serum orosomucoid concentration, level of education, smoking, alcohol intake or body mass index (table 4). After adjustment for these factors, the odds ratios in the mid and highest tertiles of IgG distribution even increased slightly. The predictive significance of IgA persisted as an 'n shaped' curve after adjusting for potential confounding factors. Further adjustments for other immunoglobulins did not materially change the risk estimates for IgG and IgA (data not shown).

The associations between serum IgG and IgA and the risk of RF positive RA were, in general, constant among various subgroups of the study population, for example, sex, age groups, and classes of potential confounders mentioned above (data not shown). Data analysis was also stratified by the time span from baseline examination until the development of RA and by RF status at baseline. Unexpectedly, the associations between serum IgG and IgA and the risk of RF positive RA were even stronger when the interval to the onset of disease was 10 years or more and RF had not been detected from the baseline serum samples (table 5).

Table 4 Relative risks (odds ratio with 95% confidence intervals, CI) of RF positive RA in middle and highest tertiles of serum IgG and IgA concentrations compared with the lowest tertiles, as adjusted for serum orosomucoid, body mass index, smoking, alcohol intake, and level of education

	Mid tertile		Highest tertile		p for trend
	Odds ratio	95% CI	Odds ratio	95% CI	
IgG	1.55	(0.81–2.97)	2.22	(1.16–4.26)	0.01
IgA	2.23	(1.14–4.36)	1.78	(0.89–3.57)	0.81

Table 5 Relative risks (odds ratios with 95% confidence intervals, CI) of RF positive RA among tertiles of serum IgG and IgA concentration by RF status at baseline and the time span from the baseline examination until the development of RA

Time span	Lowest tertile (reference group)		Mid tertile				Highest tertile			
	Number of cases	Number of controls	Number of cases	Number of controls	Odds ratio	95% CI	Number of cases	Number of controls	Odds ratio	95% CI
IgG										
< 10 years	13	45	15	48	1.06	0.45–2.47	20	46	1.56	0.68–3.57
≥ 10 years	9	46	16	39	2.10	0.81–5.45	15	33	2.30	0.89–5.93
IgA										
< 10 years	11	41	19	49	1.42	0.63–3.22	18	49	1.31	0.56–3.08
≥ 10 years	7	41	17	35	3.00	1.07–8.43	16	42	2.36	0.81–6.84
RF negative at baseline										
IgG										
< 10 years	10	26	10	35	0.73	0.27–2.01	11	29	1.00	0.35–2.84
≥ 10 years	6	36	15	29	3.31	1.10–9.97	10	26	2.11	0.67–6.60
IgA										
< 10 years	8	27	14	33	1.46	0.54–3.94	9	30	0.98	0.33–2.81
≥ 10 years	5	32	15	28	3.36	1.09–10.35	11	31	2.20	0.65–7.39

Discussion

The main finding of this work was that serum IgG was proportional to the risk of RF positive RA. A non-linear association was present between serum IgA and the risk of RF positive RA and IgM carried no predictive significance. None of the three immunoglobulins predicted the development of RF negative RA.

Polyclonally raised serum immunoglobulins are seen in association with many infective and inflammatory conditions. A rise is presumptive evidence of a response to specific antigenic stimulation, even though the identity of the antigen is often unknown. Another possibility is non-specific polyclonal B cell activation, which can result from an inherent abnormality of B cells, T cell lymphokines or other signals, such as bacterial lipopolysaccharide and similar bacterial products.¹⁶

An infection would be an obvious reason for the increase in serum immunoglobulins as a result of specific immunostimulation. Intensive research has been carried out for a possible microbiological aetiology of RA. Yet no evidence has been found for the constant presence of an infectious agent causing this disease. Another problem in explaining RA as an infectious disease is the lack of epidemiological evidence; RA does not in general cluster either in time or in place. On the other hand, the role of an infectious agent either as a trigger of the disease or as a persistent antigenic drive to inflammatory synovitis has not been ruled out. If an infectious agent plays a part in the aetio-pathogenesis of RA, one could expect that this agent would have a high prevalence in the normal population and that only a small proportion of affected people would develop RA.

Persons who develop RA may thus have an aberrant immune response to common microbial antigens or other antigens in the environment. Such response might be caused by intrinsic defects in either the T or B cell arms of the immune system. Studies of B cell subpopulations and autoantibody production in RA as well as arthritis models in animals have suggested both an intrinsic B cell defect as well as a regulatory abnormality.^{17,18} Obviously, such defects might lead to an increase in serum immunoglobulins independently of clinical symptoms of RA.

The incident cases of RA in this work were identified on the basis of their entitlement to specially reimbursed medication under the nationwide sickness insurance scheme. Most RF positive cases meet the American Rheumatism Association 1987 revised criteria for RA.¹⁹ Because the sensitivity of the sickness insurance data also exceeds 90% for RF positive RA,²⁰ it is reasonable to assume that the follow up of morbidity was sufficient for purposes of this study.

The classification of our RA cases into RF positives and RF negatives was based on information from reimbursement certificates. RF testing had been performed in several different laboratories. It is noteworthy that none of the preillness specimens from cases classified here as RF negatives were RF positive, whereas 30%

of the preillness specimens from cases classified as RF positives were RF positive. It can thus be concluded that the above classification was sufficiently accurate.

It is well established that the occurrence of RA is dependent on age and sex,²¹ while serum immunoglobulin values are likewise age and sex dependent.²² Our matched case-control study designs, however, effectively eliminated the confounding influence of age and sex. Other factors could also be associated with both immunoglobulins and RA and thus would confound comparisons. Smoking, alcohol intake, comparative weight, and the level of education have been associated with the risk of RA,^{20,23-28} and may be covariates of serum immunoglobulins.^{29,30} As adjusted for these factors, the odds ratios tended to increase.

RF positive RA may represent a single disease entity, whereas patients with clinical features of RA, but who have negative RF tests, present a classification problem. A collaborative Finnish-Russian study of patients from Heinola (Finland) and Moscow provided evidence that RF negative RA as seen in the above two centres differed in clinical and immunogenetic characteristics.³¹

Previous studies have shown that AKA can occur in RF negative cases although at a lower frequency than in RF positive cases.⁷⁻⁹ According to our experiences, however, the great majority of AKA positive RA cases are RF positive. In the present series, only two AKA positive cases developed RF negative RA. Thus, serum IgG may in general predict RA that is positive for marker antibodies of the disease.

The prevalence of positive RF reactions in preillness specimens is dependent on the period from taking of the specimens to the onset of disease; when the period is short, the prevalence is nearly the same as in the established disease,⁷ a pattern that also emerged in the work described here. Unexpectedly, IgG and IgA concentrations were already increased when the period to the onset of disease was more than 10 years and indeed the odds ratios were higher than in cases in which the period was less than 10 years. It thus seems that the increase in IgG is an earlier event than the appearance of RF and AKA and it is possible that the events reflected as increased immunoglobulin values set the autoimmune process in motion although there is no direct evidence for this. The underlying reason for the immunoglobulin increase may be an infection, but this again is only speculation. Whatever the cause may be, our findings reinforce the concept that some as yet unknown key events in the pathogenesis of RA may occur many years before the joints become affected. Perhaps the primary trigger may be traced from childhood or adolescence.^{32,33}

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