Subclass distribution of IgG antibodies to the rat oesophagus stratum corneum (so-called anti-keratin antibodies) in rheumatoid arthritis

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SUMMARY

Serum IgG, labelling the stratum corneum of the rat oesophagus epithelium, so-called anti-keratin antibodies (AKA) constitute the most specific marker for the diagnosis of rheumatoid arthritis. In this study, we investigated 31 IgG AKA-positive rheumatoid sera and 21 control sera from patients with non-rheumatoid inflammatory rheumatic diseases. The serum level of IgG1,2,3 and 4 was determined by radial immunodiffusion and the subclass distribution of IgG AKA by a three-step semi-quantitative immunofluorescence assay using standard monoclonal antibodies specific for each of the four human IgG subclasses. In the rheumatoid sera, the serum level of IgG1 was found to be significantly increased and the level of IgG2 significantly decreased with regard to the control sera, while the levels of IgG3 and 4 as well as total IgG were in the normal range. IgG1,2,3, and 4 AKA were detected in 27 (87%), 6 (19%), 4 (13%) and 11 (35%) of the 31 rheumatoid sera, respectively, and were found to be independent of the clinical and biological indices of the disease. In spite of inter-individual heterogeneity, two predominant profiles were distinguished: IgG1(alone) and IgG(1+4), which together represented 18 sera (58%). The large predominance of IgG1 AKA and the quasi-absence of IgG2 AKA suggest that the recognized antigen may be partly comprised of protein. Moreover, the high frequency of occurrence of IgG4 AKA might result from chronic exposure to the eliciting antigen, which could be a genuine autoantigen since we demonstrated that it is also present in the stratum corneum of human epidermis.

Keywords rheumatoid arthritis anti-keratin antibodies IgG subclasses

INTRODUCTION

After Young *et al.* (1979), several investigators have described by indirect immunofluorescence (IIF) in rheumatoid sera, antibodies that are able to label the stratum corneum (SC) of rat oesophagus epithelium (Johnson *et al.* 1981; Scott *et al.* 1981; Miossec *et al.* 1982; Quismorio *et al.* 1983; Ordeig & Guardia, 1984; Hajiroussou *et al.* 1985; Kataaha *et al.* 1985; Serre *et al.* 1986; Kirstein & Mathiesen, 1987). In the absence of any immunochemical evidence for the nature of the antigen they recognized, these antibodies were thought to be specific for keratins and were named anti-keratin antibodies (AKA), probably because these polypeptides are the major protein component of the SC. IgG AKA were found to be much more specific for rheumatoid arthritis (RA) than the usual biological criteria of the disease by all the investigators, and their detection was proposed as a diagnostic test for RA. In previous works (Serre *et al.* 1986; Vincent *et al.* 1989) we studied by IIF, IgG and IgM

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antibodies to rat oesophagus epithelium in a large sample of sera from patients with well-characterized rheumatic diseases, and we confirmed the high diagnostic specificity of IgG AKA in RA. Moreover, we showed that RA-specific IgG AKA labelled the rat oesophagus epithelium with a typical linear laminated pattern restricted to the SC and showed that IgM AKA as well as other anti-epithelial IgG antibodies were of no diagnostic value.

In the past decade, since highly specific monoclonal antibodies (MoAbs) directed to human G immunoglobulins 1 to 4 (IgG1–4) became commercially available, numerous works have been carried out to determine the IgG subclass profile of antibodies specific either for infectious diseases (Mergener *et al.* 1987; Nys *et al.* 1988) or for allergic diseases (Layton & Stanworth, 1984), or for various autoimmune diseases, such as bullous pemphigoid (Bird *et al.* 1986), pemphigus (Jones, Hamilton & Jordon, 1988), systemic lupus erythematosus (SLE) (Rubin *et al.* 1986; Yount, Cohen & Eisenberg, 1988), Goodpasture's syndrome (Weber *et al.* 1988) and RA in which the subclass profile of IgG rheumatoid factor (RF) has been determined (Yount *et al.* 1988). As the IgG of the four subclasses possess different immunologic properties and since it has been

suggested that preferential VDJ combinations occur with certain heavy chain C regions (Morahan, Berek & Miller, 1983), the subclass profile of specific IgG antibody can be informative of their pathogenic role in the disease as well as of the biochemical nature of the eliciting antigens.

In this study, we determined the subclass profile of the RA-specific IgG AKA by a three-step IIF assay, using standard MoAb to the human IgG1–4 previously validated in a collaborative study organized by the Human Immunoglobulin Sub-Committee of the International Union of Immunological Societies (IUIS), supported by the World Health Organization (WHO) and led by Jefferis *et al.* (1985).

MATERIALS AND METHODS

Patients and sera

The study included 5230 sera from 2573 patients which were routinely assayed by IIF for the presence of IgG AKA during the past 4 years in our laboratory. Of these patients, 650 were perfectly characterized both clinically and serologically and the 52 sera studied in this work were selected from among them.

A first group of 31 sera was randomly selected among 101 IgG AKA-positive sera, from patients with classical or definite RA (Ropes *et al.* 1958, Arnett *et al.* 1988) (22 women, nine men; age range 45–77 years, median 64). Of the 31, 27 were IgM RF-positive, i.e. they had a titre of 1/64 or more, evaluated following a modification of the Waaler–Rose method (Podliachouk, Eyquem & Jacqueline, 1958). A second group, acting as control, consisted of 21 randomly selected among 185 sera from patients with various non-RA inflammatory rheumatic diseases, previously found to be negative for IgG AKA (14 women, seven men; age range 23–76 years, median 54). This second group comprised nine cases of SLE, seven with ankylosing spondylitis, two polymyositis, two crystalline deposition diseases and one with polymyalgia rheumatica.

In addition, four sera from patients with IgG-secreting myeloma, exhibiting high levels of monoclonal IgG 1–4, respectively, were used to investigate the relative affinities of MoAb to human IgG1–4 subclasses.

All the sera were aliquoted and stored at $-80°C$ until use.

Quantification of human serum IgG and IgG subclasses

Total amounts of serum IgG were determined by radial immunodiffusion (RID) using IgG-HC NOR Partigen immuno-diffusion plates purchased from Behring (Marburg, FRG). Quantification of serum IgG1–4 subclasses was performed by RID, using monoclonal IgG1–4 RID kit from ICN (Miles, Paris, France)

Monoclonal antibodies

Among the series of 74 anti-human IgG1–4 MoAbs evaluated in the collaborative WHO/IUIS study (Jefferis *et al.* 1985), several IgG1 mouse MoAbs directed to each of the four human IgG subclasses were tested to compare their affinity by ELISA using human myeloma sera containing monoclonal IgG1–4 as adsorbed antigen.

Molar concentrations of the mouse MoAb to human IgG1–4 in ascitic fluids were determined by RID using DIFFU-GEN kit from Tago (Burlingame, CA).

As determined by RID, the human myeloma sera used as immunosorbent contained high levels of monoclonal IgG1–4

which were at least 90% of the total serum IgG. The sera were diluted in pH 9·6, 50 mM bicarbonate buffer and adsorbed on polystyrene microtitration plates (Nunc, Roskilde, Denmark) at concentrations ranging from 1 μg/ml to 200 μg/ml by incubation overnight at 37°C. The plates were stored at 4°C until use. The MoAbs to human IgG1–4 were diluted in pH 7·4, 0·8% bovine serum albumin (BSA), 0·05% Tween 20/phosphate-buffered saline (PBS) to concentrations ranging from 0·10 μg/ml to 20 μg/ml and incubated for 2 h at 20°C. Then, a peroxidase-labelled goat antibody to mouse IgG (H + L) from Tago, diluted to 1/500, was incubated for 2 h at 20°C. Between each step, the plates were rinsed in 0·4% BSA/0·05% Tween 20/PBS (pH 7·4). Specific binding was revealed with orthophenylene diamine and read at 492 nm, with a Titertek Multiskan spectrophotometer (Flow Laboratories, McLean, VA).

Among the tested MoAbs, four MoAbs specific for IgG1,2,3 and 4, respectively were found to have comparable affinities in the ELISA and were selected for use in the three-step IIF assay described below: clone HP 6070 to human IgG1 (Fc) was a generous gift of Drs A. Sarnesto & O. Makela (Jefferis *et al.* 1985), clones HP 6014 to human IgG2 (Fab), HP 6050 to human IgG3 (hinge) and HP 6025 to human IgG4 (Fc), were purchased from Biomakor (Rehovot, Israel).

In addition, an IgG1 mouse MoAb to human IgG (Fc) reactive with the four subclasses; clone HP 6017, obtained from Biomakor, was also selected to be used as a control in the three-step IIF assay.

Semi-quantitative indirect immunofluorescence.

The middle third of Wistar rat oesophagus obtained from Pel-Freeze (Rogers, AR), was frozen–embedded and stored at $-80°C$ until assay. Cryostat sections (4-μm thick) were fixed by air-drying in an airtight box containing silica-gel desiccant for 1 h at 37°C then overnight at 4°C, without additional chemical fixation.

Two-step assay. After rehydratation of sections in PBS, human sera diluted to 1/10 in PBS were incubated on slides for 30 min at 37°C in a moist chamber. The slides were then incubated for 30 min at 37°C with FITC-labelled goat F(ab) fragments to human IgG (gamma-specific) (Biosys, Compiègne, France) diluted to 1/50 in PBS.

Three-step assay. After rehydratation of sections in PBS, human sera diluted to 1/5 in PBS were incubated on slides for 30 min at 37°C in a moist chamber. Then, the slides were again incubated for 30 min at 37°C with dilutions of ascitic fluids containing the MoAb to human IgG or IgG1–4. Finally, the slides were incubated for 15 min at 37°C with FITC-labelled sheep IgG to mouse IgG (H + L) (Biosys) diluted to 1/50 in PBS. Optimal dilutions of ascitic fluids with MoAb to IgG and to IgG1,2,3, and 4 were found to be 1/80, 1/1200, 1/420, 1/270 and 1/300, respectively, corresponding in all cases to a final MoAb concentration of 15 μg/ml.

In both assays the slides were rinsed twice after each step, for 3 min in PBS/Tween 20, then 3 min in PBS in the two-step assay and for 1 min in PBS in the three-step assay. The slides were mounted with Fluoprep medium (Bio-Mérieux, Lyon, France). Observations were made under an Olympus BH2 (Tokyo, Japan) microscope with u.v. epi-illumination and photographs were taken with an Olympus OM4 camera.

The fluorescence intensity of the SC of the cornified stratified squamous epithelium of the rat oesophagus was

Table 1. Median concentration (range) of IgG subclasses and total IgG, determined by radial immunodiffusion in RA sera and in control sera

	Concentration of serum immunoglobulin (g/*l*)				
	RA sera		Control sera		*P**
IgG1	12·0	(5·1–21·0)	8·3	(5·4–18·0)	0·001
IgG2	1·9	(0·3–6·5)	3·5	(0·5–6·9)	0·005
IgG3	1·1	(0·4–2·3)	0·9	(0·3–1·6)	NS
IgG4	0·6	(0·03–2·6)	0·5	(0·03–2·5)	NS
Total IgG	13·7	(7·5–23·4)	13·1	(7·5–21·1)	NS

NS, not significant
* Mann–Whitney *U*-test.

estimated by two readers uninformed of the clinical context, according to a semi-quantitative scale from 0 to 4 (0·25 step). The reliability of this semi-quantitative evaluation has been validated in previous works (Serre *et al.* 1986, 1987; Vincent *et al.* 1989). All the sera, previously selected as positive for IgG AKA, were first verified together in the IgG-specific two-step IIF assay. Then the sera were twice assayed in three-step IIF assays, following two different designs, transversal and longitudinal, two slides per serum being tested in each assay. In a first series of assays (transversal design), the IgG AKA and the IgG1–4 AKA were investigated simultaneously in each serum, four sera being tested in each assay. In a second series of assays (longitudinal design), all the sera were tested simultaneously, in a separate assay for each subclass. The comparability and the reproducibility of the double-blind reading were insured by internal controls avoiding major inter-reader and inter-assay variations. The consistency of the eight estimations/specificity per serum (two assays × two slides per serum in each assay × two readers) was assessed before the estimation of each reader was averaged. Then, the estimations of the readers were summed in order to obtain a titre-like value ranging from 0 to 8. The fluorescence intensity of the stratum spinosum (SS) and the stratum basale (SB) was evaluated in the same way. In addition, the labelling pattern, i.e. cytoplasmic or pericellular, linear, laminated, diffuse, microgranular, speckled or flaky, was analysed in each epithelial compartment.

Statistical analysis
Statistical analyses were performed using home-made software on a BM60 computer (Bull-Micral, Massy, France). Mean differences were tested with the Mann–Whitney *U*-test and correlations were sought using Spearman's rank correlation coefficient. Percentages were compared using the χ^2 test.

RESULTS

Control sera
The levels of serum IgG and IgG1–4 are given in Table 1. In the group of non-RA sera, a good correlation was found between the amount of total IgG and the amount of IgG1 (Spearman rank correlation coefficient = 0·671, $P < 10^{-3}$) while no correlation was found between total IgG and the other IgG subclasses.

In the two-step IIF assay, the 21 control sera were found to be negative for IgG AKA, i.e. their titre-like value was always

lower than the positivity threshold (2.00) determined in a previous work (Serre *et al.* 1986). When investigated for IgG AKA in the three-step IIF assay, the control sera produced a background fluorescence on the SC, with a titre-like value ranging from 0·25 to 1·50 on the semi-quantitative scale. These sera were then tested for IgG1–4 AKA in order to determine the threshold above which the presence of specific AKA could be affirmed. The maximum titre-like values obtained were 1·25 for IgG1, 1·50 for IgG2, 1·75 for IgG3 and 1·25 for IgG4 AKA. Since the four anti-human IgG subclass MoAbs we selected were found to be of similar affinities and used at the same molar concentration, we chose 2·00 (maximum titre-like value observed with the control sera + one step of the semi-quantitative scale) as the threshold for the detection of IgG1–4 AKA in the three-step IIF assays.

The other epithelial compartments (SS, SB) showed low cytoplasmic background fluorescence, regardless of the tested IgG subclass. Non-disease-specific IgG antibodies giving a pericellular labelling of the SB were shown to only belong to the IgG2 subclass. In the same way, a serum from a patient with SLE exhibited a high titre-like value of anti-nuclear antibodies which were detected in all four IgG subclass assays.

Rheumatoid sera
As shown in Table 1, the median serum concentration of IgG1 was found to be significantly higher in RA sera than in control sera, and the median serum concentration of IgG2 significantly lower. As in control sera, a close correlation was found between the amount of total serum IgG and the amount of serum IgG1 (Spearman rank correlation coefficient = 0·876, $P < 10^{-3}$) while no correlation was found between total serum IgG and the other serum IgG subclasses.

All the rheumatoid sera which had been previously selected as positive for IgG AKA, were verified in the two-step IIF assay and their positivity confirmed. In the three-step IIF assay for IgG antibodies the minimum titre-like value we found was greater than the maximum titre-like value obtained with the control sera (2·00 *versus* 1·50), attesting for the presence of detectable specific IgG AKA in all the tested RA sera. Moreover, a close correlation was found between the IgG AKA titre-like values estimated in the two-step assay and in the three-step assay (Spearman rank correlation coefficient = 0·800, $P < 10^{-3}$).

When specific antibodies were found to be present with a high titre-like value, the rat oesophagus epithelium was labelled with a linear laminated pattern restricted to the SC. This typical pattern, produced by IgG AKA in the two-step as well as in the three-step IIF assay, was also found when RA sera were tested for IgG1–4 antibodies. As with non-RA sera, SS and SB showed low, non-specific cytoplasmic background fluorescence, whatever the IgG subclass tested. A non-disease-specific occasionally intense pericellular labelling of the SB was observed with some rheumatoid sera when tested for IgG antibodies. The antibody responsible was found to be IgG2 antibody but never IgG1,3 or 4 antibody. Figure 1 shows the patterns of labelling obtained with an IgG AKA-positive RA serum tested for IgG in the two-step IIF assay and for IgG1–4 in the three-step IIF assays.

IgG1 AKA were found to be present in 27 of the 31 RA sera (87%) and predominant in a large majority of them (n = 23; 74%). When detectable IgG AKA belonged to only one subclass, they were found to be IgG1 AKA (*n* = 10; 32%) and

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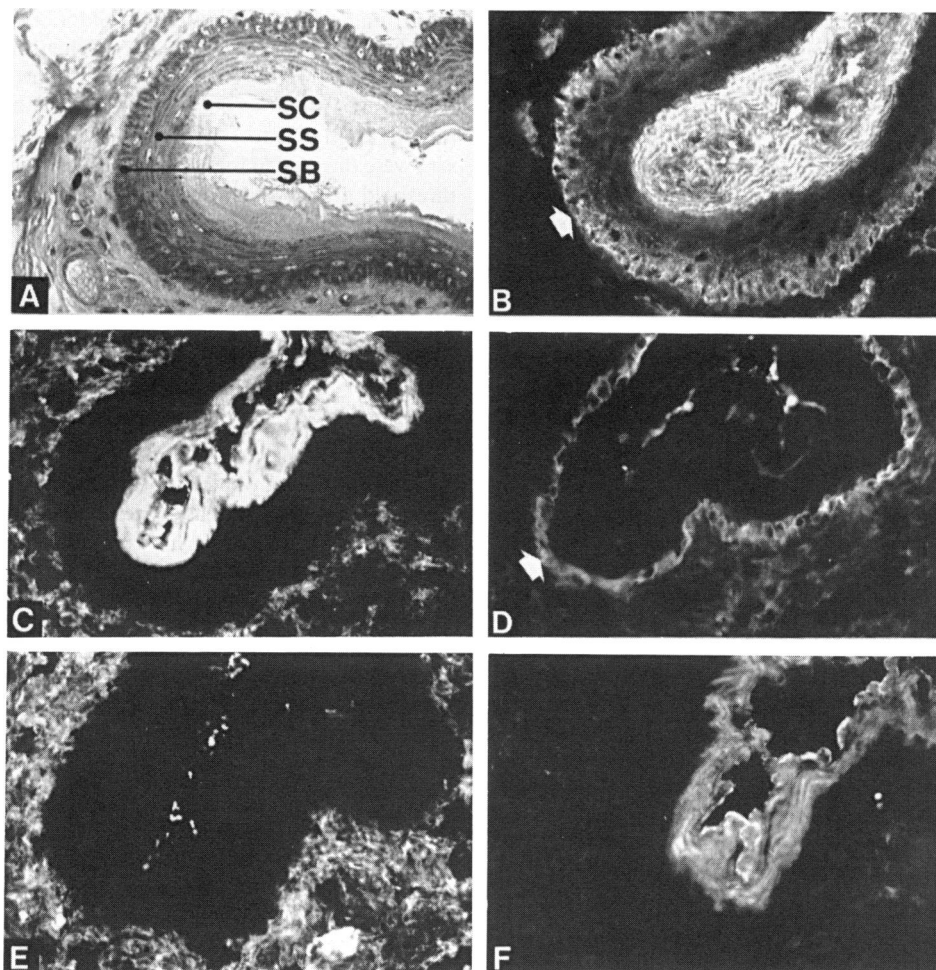
Fig. 1. Histology of the rat oesophagus and patterns of labelling produced by a typical RA serum (no. 27 in Table 2). A, Histological structure of the rat oesophagus epithelium, SC, stratum corneum; SS, stratum spinosum; and SB, stratum basale; B, IgG antibodies detected in the two-step IIF assay. C,D,E,F, IgG1,2,3 and 4 antibodies respectively, detected in the subclass-specific three-step IIF assays. RA-specific IgG AKA (B), giving a linear laminated labelling pattern of the SC were found to be IgG1 AKA (C) and, to a minor degree, IgG4 AKA (F). Non-disease-specific IgG antibodies (B, arrow), giving a pericellular labelling of the SB were found to be exclusively IgG2 antibodies (D, arrow). Background fluorescence of the chorion (C, D, E) is probably due to the cross-reactivity of the labelled anti-mouse antibody to rat IgG.

when IgG AKA belonging to the other IgG subclasses were found to be present, they were always associated with IgG1 AKA. Intense labelling of the SC was found with some sera when tested for IgG3 and particularly for IgG4 AKA but these subclasses were found to be predominant in only 1 (3%) and 3 (10%) sera, respectively. About one-third of the sera ($n=11$; 35%) contained IgG4 AKA while IgG2 and IgG3 AKA were only detected in six (19%) and four (13%) sera, respectively (Table 2). Only four of the 31 sera (13%) were found to be negative for IgG AKA in the four IgG subclass assays. Although large interindividual variations were found in the IgG AKA subclass profiles (Fig. 2), two predominant profiles were distinguished: IgG1 AKA alone and IgG(1+4) AKA which together represented 18 (58%) sera.

As shown in Table 3, the titre-like value of IgG AKA was found to be correlated to each IgG AKA subclass titre-like value. In contrast, the titre-like value of IgG AKA and of IgG1–4 AKA were found to be independent of the total serum IgG and serum IgG1–4, respectively. Moreover, the serum levels of IgG and IgG1–4 as well as the titre-like values of IgG AKA and of

IgG1–4 AKA were found to be independent of age, sex and IgM RF titre.

DISCUSSION

The median serum IgG concentration we determined in RA sera was very similar to the median IgG concentration we found in non-RA sera and both of them were in the normal range (Jiménez, López-Trascasa & Fontán, 1988). In contrast, the median serum IgG1 concentration of RA sera (12 g/l) was largely beyond the 97·5 percentile (10·2 g/l) determined with the same RID method by Djurup *et al.* (1988) and by French & Harrison (1984) in 200 and 172 sera, respectively, from healthy adults. Moreover, the median IgG1 concentration in RA sera was found to be significantly higher than in non-RA sera but was balanced by the significantly lower concentration of IgG2, resulting in similar median serum IgG concentrations in the two groups of patients. Both for RA sera and control sera, the median serum concentrations of IgG2,3, and 4 antibodies were included in the normal ranges reported in previous studies
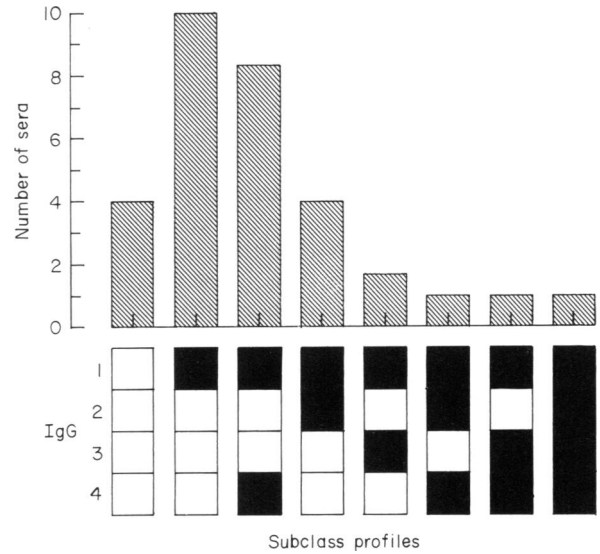
Table 2. Semi-graphic representation and distribution parameters of IgG AKA and IgG1–4 AKA titre-like values in RA sera

Serum no.	Two-step IIF assay	Three-step IIF assay				
	IgG	IgG	IgG1	IgG2	IgG3	IgG4
12	*	*	—	—	—	—
24	*	*	—	—	—	—
19	*	*	—	—	—	—
9	*	**	—	—	—	—
7	***	**	*	—	—	*
10	*	**	*	—	—	—
20	*	**	*	—	—	—
15	*	**	*	—	—	—
4	*	**	*	—	*	—
2	**	**	*	*	—	—
3	**	**	**	—	—	—
17	**	**	*	—	—	—
11	**	**	**	—	—	—
6	*	***	***	—	—	—
27	****	***	****	—	—	**
5	**	***	**	—	—	*
8	***	***	***	—	—	***
22	***	***	**	—	—	—
13	***	***	**	—	—	—
30	***	***	***	—	*	—
28	**	***	**	—	—	**
1	**	***	**	—	—	***
26	**	***	***	—	—	*
16	****	****	**	—	—	*
23	****	****	**	—	**	***
21	***	****	***	*	—	*
18	****	****	***	*	—	—
29	****	****	***	*	*	****
31	****	****	**	—	***	—
14	****	****	****	—	—	—
25	****	****	*	—	***	—
Minimum	2·25	2·00	1·00	0·00	0·25	0·25
Median	3·75	4·25	3·50	0·75	1·00	1·75
Maximum	6·50	6·00	5·50	2·75	4·75	5·00

—, <2·00; * ≥2·00, <3·00; ** ≥3·00, <4·00; *** ≥4·00, <5·00; **** ≥5·00. Titre-like values were appreciated by the semi-quantitative evaluation of the SC fluorescence intensity (arbitrary units from 0 to 8).

Fig. 2. Distribution of the 31 rheumatoid sera within the eight observed IgG AKA subclass profiles among the 16 possible. □, IgG1,2,3 or 4 AKA titre-like value <2·00; ■, IgG1,2,3 or 4 AKA titre-like value ≥2·00.

Table 3. Correlations between IgG AKA and each of the IgG1–4 AKA titre-like values

AKA	r	P
IgG/IgG1	0·734	$<10^{-3}$
IgG/IgG2	0·435	0·015
IgG/IgG3	0·675	$<10^{-3}$
IgG/IgG4	0·394	0·028
IgG/IgG1+2+3+4*	0·853	$<10^{-3}$

* Correlation between IgG AKA and the sum of the IgG1,2,3 and 4 AKA titre-like values.

r Spearman rank correlation coefficient.

(French & Harrison, 1984; Djurup *et al.* 1988; Jiménez *et al.* 1988). Whether the increase of serum IgG1 is characteristic for RA in general or for a particular form of RA, remains to be investigated.

IIF is the only method which has been described to date, to investigate IgG AKA. Because the fluorescence intensity is dependent on the number of antibodies complexed to their tissue antigens, IIF allows not only the detection of specific antibodies, but also a semi-quantitative evaluation of their level, i.e. titre-like value (Young *et al.* 1979; Hajiroussou *et al.* 1985). However, to obtain reliable results, such a method requires rigorous reproducible conditions of assay and reading. Moreover, to infer the subclass profile from the titre-like values of the

respective IgG1–4 AKA, the comparability is essential not only between the sera for a given subclass, but also between the subclasses for a given serum.

Polyclonal antibodies to human IgG1–4 even when highly purified, may exhibit cross-reactivity resulting from the biochemical and structural proximity of the four human IgG subclasses. Monoclonal reagents, although they are by nature more likely to be monospecific, may show varying degrees of specificity and affinity which, moreover, can also depend on the type of immunoassay in which they are used (Reimer *et al.* 1984). The MoAbs we chose were found to be monospecific in solid-phase immunoassays (Jefferis *et al.* 1985) and we showed in an ELISA using monoclonal myeloma IgG as immobilized antigens that they had comparable apparent affinities for their respective antigens. Since the five chosen MoAbs to human IgG and IgG1–4 were used at the same molar concentrations in the three-step IIF assays, we can assume that comparison of the IgG and IgG1–4 AKA titre-like values is valid. Evidence for the validity of the semi-quantitative method we used to appreciate

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the relative titre-like values of IgG1–4 AKA was given by the very close correlation we found between the titre-like value of IgG AKA and the sum of the titre-like values of IgG1–4 AKA (Table 3). Lastly, the low frequency and the low titre-like value of the IgG2 AKA are unlikely to be due to a lack of sensitivity of the MoAb HP 6014, since in some RA sera as well as in some control sera we detected the presence of non-disease specific IgG2 antibody producing an intense pericellular labelling of the SB which can be regarded as an internal control for the IgG2 assay sensitivity (Fig. 1). However, some discrepancies were found between the estimations of the relative titre-like values of IgG AKA and IgG1–4 AKA. Indeed, although the detection threshold was objectively determined, four out of 31 rheumatoid sera were found to be negative for IgG1–4 AKA, while the same sera exhibited significant titre-like values of IgG AKA in the two-step and in the three-step IIF assay (Fig. 2). In these cases, AKA belonging to one or several IgG subclasses were necessarily present but their titre-like value remained under the positivity threshold we chose for the semi-quantitative evaluation. Therefore, a negative result for one IgG AKA subclass must not be considered separately, but as a component of the IgG subclass profile.

The results of the IgG subclass-specific IIF assays we present in this study, which constitute to date the first description of the subclass profiles of IgG AKA in RA, could be summarized as follows. Firstly, in spite of the absence of a strict restriction of expression to only one subclass, IgG1 AKA was found to be largely predominant and IgG2 AKA, quasi-absent, in terms of frequency of occurrence as in terms of titre-like value, independently of the serum concentrations of the related IgG subclasses. Secondly, the comparison between the titre-like values of IgG1–4 AKA allows two predominant profiles to be distinguished: IgG1(alone) and IgG(1 + 4) which together represent more than half of the 31 RA sera. Lastly, large inter-individual heterogeneity was found among the 31 analysed subclass profiles, without significant correlation with the clinical or biological indices of the disease.

No immunochemical evidence has been reported on the nature of the antigens recognized by IgG AKA. Moreover, they are, in spite of their name, clearly different from the autoantibodies to epidermal cytokeratins we described both in all the normal human sera and in rheumatoid sera (Serre *et al.* 1986, 1987). Nevertheless, the determination of the subclass profile of IgG antibodies could contribute towards the knowledge of the antigens they recognized. Indeed, protein antigens were found to be responsible for the predominant production of IgG1 antibodies (Stevens *et al.* 1983; Schatz *et al.* 1988) while IgG2 antibodies seem to be more specifically elicited by polysaccharidic antigens (Siber *et al.* 1980; Barret & Ayoub, 1986), glycolipidic antigens (Nys *et al.* 1988) or glucidic fraction of glycoprotein antigens (Schatz *et al.* 1988). A possible explanation for the existence of isotype-restricted idiotypes, also reported by Capra & Kehoe (1975), is that the constant region (CH1 domain) of the immunoglobulin influences the folding of the variable region and hences the expression of idiotype (Morahan *et al.*, 1983). Therefore, the quasi-absence of IgG2 AKA and the large predominance of IgG1 AKA compared with other subclasses, strongly suggest that the recognized antigen may be partly comprised of protein.

Although IgG4 is only 1–7% of the total serum IgG (4% in this study), IgG4 AKA were detected, sometimes with high titre-

like values, in more than a third of the IgG AKA-positive rheumatoid sera. It has been shown that the production of specific IgG4 antibodies may be induced by allergens (Aalberse, van der Gaag & van Leeuwen, 1983; Layton & Stanworth, 1984) and also by autoantigens (Bird *et al.*, 1986; Jones *et al.*, 1988; Fukuma *et al.*, 1989). Moreover, as suggested for IgG RF (Cohen *et al.*, 1986; Yount *et al.*, 1988), specific IgG4 antibody production might result from chronic exposure to the eliciting antigen. Thus, a similar phenomenon could be at the origin of the high levels of IgG4 AKA we found in RA sera since, not only are the IgG subclass profiles of both IgG RF and IgG AKA very similar (with, in both cases, a predominance of IgG1 and IgG4), but also, we previously demonstrated that the antigen recognized by IgG AKA is a genuine autoantigen also present in the SC of human epidermis (Serre *et al.*, 1986).

IgG AKA constitute the most specific serologic marker for RA. Nevertheless, about 1% of non-RA sera also label the SC of rat oesophagus. In these cases, either the labelling pattern can be distinguished from the specific IgG AKA pattern, avoiding the sera involved being considered as positive, or it cannot be distinguished, leading to an incorrect diagnostic. In the latter case, the study of the IgG subclass profile might contribute toward being able to distinguish between true (RA) and false (non-RA) positive sera.

In spite of their high diagnostic value and although they seem to be associated with more active and/or severe forms of the disease (Vincent *et al.*, 1989), no pathogenic role has been reported to date for IgG AKA in RA. In order to clarify the significance of the IgG AKA subclass profile and subsequently the possible implication in RA pathophysiology of these very specific antibodies, further investigation is necessary on larger samples and on longitudinal series. Indeed, the heterogeneity of the IgG AKA subclass profile might reflect either variations that could occur in the course of disease evolution or the intrinsic heterogeneity of RA both clinically and biologically. These hypotheses, which are not mutually exclusive, raise the possibility that the study of the subclass profiles may enhance the diagnostic and/or prognostic value of IgG AKA in RA.

ACKNOWLEDGMENTS

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