Veterinary Immunology and Immunopathology, 19 (1988) 259-271
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Antibodies to Immunoglobulin-G in Dog Sera, Synovial Fluids and Aqueous Humor: a Comparative Study of Rheumatoid Factor Assays, Suitable for Routine Application

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(Accepted 17 September 1987)

ABSTRACT

Bernadina, W.E., Van Kol, P.J. and Willemse, A., 1988. Antibodies to immunoglobulin-G in dog sera, synovial fluids and aqueous humor: a comparative study of rheumatoid factor assays, suitable for routine applications. *Vet. Immunol. Immunopathol.*, 19: 259-271.

The incidence of anti-IgG antibodies (rheumatoid factors, RF) in body fluids (sera, synovial fluids and aqueous humor) selected from 62 normal and 275 diseased dogs was studied. Fluids were assayed by canine versions of standard agglutinating and/or precipitating RF assays with routine application in human practice. The number of RF detected by dog IgG-coated particles was substantially higher by latex fixation test (LFT) than by modified Rose-Waaler (RW) test (61/144 vs. 14/144). This did not result from false positives by LFT since latex activity was completely inhibited by aggregated dog IgG. Some evidence is presented indicating that results obtained by standard RW in particular, but also those obtained by standard LFT, might be improved by modifying testing conditions currently used. Body fluids were further studied for the presence of precipitins to aggregated dog IgG in 0.6% agarose (gel precipitation test (GPT)). The frequency of RF was higher by GPT than by LFT, both in normal control fluids (for sera 26/52 vs. 19/52) and patient material (for sera 135/197 vs. 95/197). Thus, the canine RF appear to be a serum component with an unexpectedly high frequency in both normal and diseased dogs, but grossly underestimated by the recommended routine RF assays based on agglutination. The GPT, which combines a superior detection rate of theoretically also agglutinating RF with an inability to detect RF quantitatively, seems an ideal RF 'indicator' test to dictate improvements to the quantitative LFT/RW assays so as to facilitate RF detection at clinically relevant concentrations. Thus optimized, RW/LFT would provide the optimal detection apparatus for the ultimate isolation of the relevant 'RF' repertoire present, for comparative studies aimed ultimately at unraveling the etiopathogenesis of the 'real' RF.

0165-2427/88/$03.50

INTRODUCTION

The presence of so-called rheumatoid factors (RF) and/or anti-gamma globulins is a feature of various diseases, both in man and animal species (Theophilopoulos and Dixon, 1980; Goudswaard, 1981; Fye and Sack, 1984; Theofilopoulos, 1984). In man the presence of circulating RF is one of the major criteria helpful in making the diagnosis of rheumatoid arthritis (RA). Although IgG-directed autoimmunity itself may not be the cause of RA, these autoantibodies have been implicated in the pathogenesis of many disease manifestations in human RA (Harris, 1981; Jones and Orlans, 1981). Of special interest is the recent suggestion that anti-gamma globulins, including all immunoglobulin classes of RF (i.e. IgG-, IgM- and IgA-RF) may be involved in both the destruction and self-perpetuation stage of RA (Pope and Talal, 1985).

In dogs, RA has also been well documented (e.g. Alexander et al., 1976; Pedersen et al., 1976). Moreover, there is evidence to indicate that the dog mimics the human disease, including the immune-mediated conditions (e.g. Newton et al., 1976; Pedersen and Pool, 1978). This finding thus permits relevant animal studies, offering more choice for studying pathogenicity of RA-associated anti-gamma globulins compared to human studies.

During the performance of some preliminary studies, we made two observations that have led us to believe that dog studies might also help in understanding the mechanism by which autologous IgG becomes immunogenic. (a) Sera from clinically healthy normal dogs with no history of disease/fever, but manifesting serum levels of anti-streptolysins, often exhibit anti-IgG activity. Pre-treatment of these sera with aggregated IgG (self or non-self) substantially inhibits their anti-IgG activity. (b) In a quantitative laser nephelometric system using various homologous antisera obtained from either diseased (i.e. with chronic dermatitis and leishmaniasis) or normal healthy dogs (i.e. used for preparing amboceptor), false positive readings were observed with control sera. These reactions could all be abolished, either by prior incubation of the homologous antisera with aggregated dog IgG, or by performing the test using untreated antisera on IgG-depleted control test specimens. Collectively, these findings seem to point to the presence in dog sera of a large RF repertoire awaiting further immunopathological characterisation. However, the appropriate methodology for their detection for subsequent isolation has not yet been evaluated. To that end, we assayed both normal and diseased biological fluids for anti-IgG activity by canine versions of routinely applied human RF assays. That is, the modified Rose-Waaler test (RW), the latex fixation test (LFT), and the gel precipitation test (GPT). We here report the results of this investigation and also discuss possible manoeuvers by which the usefulness of the quantitative RF assays employed can be increased.

MATERIALS AND METHODS

Subjects

The study comprised 275 diseased and 62 clinically healthy normal donor dogs belonging to the breeds most commonly seen in The Netherlands, with ages ranging from 5 months to 15 years. All patients were examined and diagnosed at the University Clinic for Small Animals in Utrecht. They included: 69 dogs with arthritis (26 with rheumatoid-like disease (RLD)), 46 dogs with skin disorders, 17 dogs with leishmaniasis, 58 dogs with renal diseases, and 85 for convenience called 'non-defined diseased' dogs.

Canine sera

Blood was obtained by venepuncture. After incubation at room temperature for 1–2 h, sera were removed from the clots by centrifugation at 4000 *g* for 10 min at 4 °C. Sera were used immediately or stored at −70 °C until required.

Synovial fluids

Samples were obtained by puncture of distended joints in the case of arthritis, or by puncture of the knee joints in other cases. The synovial fluids were centrifuged at 11 800 *g* for 10 min at 4 °C and either used immediately or stored at −70 °C until used. Prior to use in RF assays, synovial fluids were treated with hyaluronidase, as described by Male and Roitt (1981), to facilitate their manipulation.

Aqueous humor

Aqueous humor was obtained by puncture into the anterior chamber of the eyes immediately after euthanasia. After clearance by centrifugation, the aqueous humor was stored at −70 °C until used.

Preparation of dog IgG

IgG was prepared from pooled, latex-negative normal dog serum by elution from DEAE cellulose (Whatman DE-52) with 0.02 *M* phosphate buffer, pH 8.0, followed by filtration through AcA 44 (LKB, Sweden) in PBS (phosphate-buffered saline), pH 7.2 (flow rate 15 ml/h). The second protein peak was pure IgG as found by immuno-electrophoresis and double immunodiffusion analysis with both heavy-chain specific anti-dog IgG and anti-dog whole antiserum. This IgG was rendered RF-free by successive passages over immunoabsorbents containing dog IgG bound to Sepharose 4B beads, and subsequently

concentrated to 60 mg/ml. Dilutions of this stock solution were used to prepare assays for the detection of RF.

Aggregated dog IgG

Heat-aggregated dog IgG was prepared by incubation of 3 ml of a concentrated IgG solution (40 mg/ml) at 63 °C for 30 min. The resultant aggregated IgG was separated from non-aggregated IgG by filtration through AcA 34 (Schalèn and Christensen, 1977). The peak containing aggregated IgG was concentrated to 7 mg/ml and stored at −70 °C until used in both gel diffusion experiments and inhibition studies.

Preparation of IgG-linked red cells

'Universal' dog donor blood (=group A^- (negative)) in Alsever's solution was supplied by the University Clinic for Small Animals. Five-hundred μl of packed washed (three times with 0.9% NaCl) group A^- red cells were added to 1.5 ml of aggregated dog IgG (1 mg IgG/ml saline) in 3.5 ml of normal saline. Twenty-five μl of freshly prepared 0.05 *M* chromic chloride in saline were then added dropwise, with constant mixing during addition. Following incubation for 1.5 h at 37 °C (waterbath), with mixing every 15 min, the IgG-coupled cells were washed three times with saline (0.9% NaCl) and once with 0.5% heat-inactivated fetal calf serum in PBS-Ca·Mg[1]. The IgG-erythrocytes were then adjusted to a 10% (*v*/*v*) suspension in PBS-Ca·Mg and stored at 4 °C for 3 days maximally. Coupling efficiency was tested by titration with rabbit anti-dog IgG antiserum (heat-inactivated and absorbed with dog red cells). The agglutinating titre of our IgG-linked red cells remained the same for 3 days.

Preparation of Sepharose 4B–dog IgG column

Dog IgG, freed from RF, was coupled to CNBr-activated Sepharose 4B (Pharmacia, Sweden) according to the directions of the manufacturer, using 5–10 mg IgG to 0.3 g (dry weight) Sepharose.

Sample handlings

The following measures were taken to minimise non-specific binding of samples to RF reagents and to ascertain specificity of observed bindings to IgG-coupled ligands: (a) all test fluids were heat-inactivated; (b) test fluids to be assayed by RW were also absorbed with sheep red blood cells; (c) to specimens to be applied to the RF immunoabsorbent, BSA (bovine albumin powder

[1]PBS-Ca·Mg = PBS + 0.132 g $CaCl_2 \cdot 2H_2O$/l + 0.1 g $MgCl_2 \cdot 6H_2O$/l, pH 7.2.

Sigma) was added to a final concentration of approximately 6 mg/ml. As regards the binding to IgG-coupled ligands, two procedures were used in inhibition studies to ascertain specificity of reaction for all fluids adsorbing to Sepharose 4B-IgG (cf. Table 1) and for 91 test fluids adsorbing to the latex canine RF reagent. In one set of experiments, fluids (serially diluted; in duplicates) were pre-incubated with aggregated RF-free dog IgG containing 1 or 5 mg IgG/ml for 10 min. These mixtures were then immediately assayed for remaining anti-IgG activity by LFT, this activity denoting non-specific agglutination (in our hands, for sera at dilutions $<1:30$). In another set of experiments, each specimen manifesting anti-IgG activity was incubated with an appropriate amount of RF-neutralising aggregated RF-free IgG (Harboe and Ingild, 1983). Following incubation at 37°C for 1 h and overnight at 4°C and then removal of precipitates (11 800 *g* at 4°C), test specimens were immediately reapplied to IgG-ligand for assessing remaining activity. All samples thus studied for specificity of observed reactions (cf Tables 1, 2) were found to specifically adsorb to IgG-coated ligands.

Rheumatoid factor assays

Modified Rose-Waaler (RW) test

Sheep red cells (SRBC) were sensitised, using one half of the minimal haemagglutinating dose of dog anti-sheep red cell antiserum. Fifty μl of a 2% cell suspension was added to sample titrations in PBS-Ca·Mg in 50 μl volumes, set up in microtiter plates (sera 1:10, 1:20, and 1:40; aqueous humor undiluted, 1:5 and 1:10; synovial fluids 1:5 and 1:10). Sensitised red cells incubated with PBS-Ca·Mg, and normal rabbit serum as well as RF-free dog serum (see preparation of RF-free dog IgG) served as negative controls. Sensitised red cells incubated with rabbit IgG anti-dog IgG served as a positive control.

Passive haemagglutination (HA) test

The HA was performed essentially as described for the RW, using 50 μl of 2% IgG (chromic chloride)-coupled A^- dog red cells. Controls were the same as used in the RW.

Latex fixation test (LFT)

The LFT was performed on slides using 50 μl of test fluid to react with 50 μl of latex RF reagent. After agitating for up to 2 min, agglutination was read by comparing with positive (rabbit anti-dog IgG) and negative controls. Test fluids were considered positive by LFT when, also at retesting, the latex reagent was aggregated at the following dilutions: sera, $\geqslant 1:30$; aqueous humor, either undiluted or diluted; synovial fluids, diluted 1:5 or 1:10. All fluids were retested after 24–48 h. Bias as well as operator-dependency of the LFT reading

were avoided at retesting by using fresh observers who had no knowledge of LFT results at first testing.

Gel precipitation test (GPT)

Double immunodiffusion experiments were performed essentially as described by Schalèn and Christensen (1977). Briefly, 20 μl of undiluted test fluid and 7 μl of aggregated IgG (7 mg/ml) were allowed to diffuse into 0.6% agarose for 2 days at room temperature, after which plates were stained and read.

Affinity chromatography

Samples in BSA (6 mg/ml sample) were applied to the anti-IgG immunoabsorbent. Adsorbed anti-IgG was eluted with 0.1 *M* glycine HCl, pH 2.6, containing 0.5 *M* NaCl, and neutralised immediately with Tris, pH 9.5. After dialysis against PBS, pH 7.2, LFTs were performed on this material.

RESULTS

Specificity of latex RF reagent

Comparative binding of 26 serum samples to Sepharose–IgG was used to determine whether our latex canine RF reagent was really detecting anti-immunoglobulin G. These control samples included the latex-negative sera used to prepare RF-free dog IgG ($n=10$), heavy-chain specific rabbit ($n=1$) and goat ($n=1$) anti-dog IgG, and both latex-positive and latex-negative diseased sera ($n=14$).

TABLE 1

Latex fixation test results of sera vs. their capacities to bind to dog IgG–Sepharose

Samples	Category	No. of sera tested	No. of sera positive by LFT[a]	No. of sera adsorbing to Sepharose	No. of eluates positive by LFT[b]
Anti-dog IgG (rabbit)	—	1	1	1	1
Anti-dog IgG (goat)	—	1	1	1	1
Sera	Normal	10	0	6	0
Sera	Patient	14	5	8	8(5)[c]

[a]LFT was performed with a batch of RF reagent prepared with dog IgG.
[b]LFT was performed with a batch of RF reagent prepared with RF-free dog IgG.
[c]Value in parentheses denotes the number of sera also designated sero-positive prior to the application to IgG–Sepharose.

Between applications, the column was extensively washed with PBS, pH 7.2. The results show that six of the 10 normal sera adsorbed to IgG–Sepharose (Table 1) thus suggesting the presence of antibodies to IgG. It was noted that these antibodies were not identified by LFT. Three diseased serum samples showed adsorption to IgG–Sepharose while failing to agglutinate the latex RF reagent. However, after elution from the column, these samples were all found to be positive with both types of latex RF reagents (shown only for one). This finding is suggestive of the presence of hidden RF activity in at least three of the 14 diseased samples at first testing by LFT. It was noted that no single serum sample, designated latex-positive prior to application to IgG–Sepharose, failed to adsorb to the IgG-column. There were no false positive readings since reactivity with IgG-ligand, in all cases, could be abolished by pre-incubation with aggregated dog IgG (see Materials and Methods, sample handling). Thus, agglutination of the latex canine RF reagent used was due to anti-dog IgG antibodies present.

Comparison of results obtained by LFT with those obtained by RW and by HA of IgG-coupled A^- red cells

Test fluids, all heat-inactivated, and aliquots of these additionally absorbed with sheep red cells for the performance of the RW, were screened at dilutions mentioned in Materials and Methods. Results indicate identical fluid reactivity by both the RW and the HA (Table 2). Agglutination titres (data not shown) were also similar. Fifty-two of the 122 patient sera examined were positive by LFT, whereas only 12 of these 122 were sero-positive by the other two assays.

TABLE 2

Comparison of the LFT with both the RW and the HA of dog A^- red cells linked with aggregated dog IgG

Test fluid	Category	No. of fluids	No. of fluids designated positive by the		
			Rose-Waaler test (modified)	Passive HA test	Latex fixation test
Sera	Normal	14	0	0	0 (–)[a]
	Patient	122	12	12	52 (8)
Synovial fluids	Normal	10	0	0	0 (–)
	Patient	5	1	1	5 (1)
Aqueous humor	Normal	8	0	0	0 (–)
	Patient	17	1	1	4 (1)

[a]Values in parentheses denote the number of fluids also positive by the Rose-Waaler test.

TABLE 3

Anti-IgG antibodies as detected by latex fixation and precipitation of aggregated dog IgG in 0.6% agarose

Test fluid	Category	No. of fluids tested	No. precipitating aggregated dog IgG	No. agglutinating latex-fixed dog IgG
Sera	Normal	52	26	19 (10)[a]
	Patient	197	135	95 (76)
Synovial fluids	Normal	10	0	0 (–)
	Patient	25	4	18 (2)
Aqueous humor	Normal	8	0	0 (–)
	patient	19	2	4 (0)

[a]Values in parentheses denote the number of fluids also positive by the GPT.

Of these twelve sero-positive sera, 8 were also detected by LFT. A similar pattern of reactivity was observed for the synovial fluids and aqueous humor from patients examined.

Comparison of the LFT and the GPT

As reported above, the latex fixation test compared favourably to the other tests employed. We therefore considered the use of the LFT for screening larger numbers of dog fluids, including more normal specimens. Simultaneously, the precipitating capacities of these samples were measured by GPT. Comparative results showed that substantially more sera were positive by GPT than by LFT, whereas for the ('diseased') samples of the other fluids examined, the reverse was found (Table 3). As can be seen, a great part of the precipitating anti-IgG factors were incapable of RF-latex agglutination under the testing conditions used. On the other hand it was also noted that substantial numbers of samples that were positive by LFT escaped detection by GPT.

DISCUSSION AND CONCLUSIONS

Anti-dog IgG factors had come to be recognised as the canine counterparts of human RF when anti-gamma globulins were detected in dogs with RA according to the criteria of the American Rheumatism Association (Newton et al., 1976; Pedersen et al., 1976). Subsequent studies indicated, however, that anti-gamma globulins might also be detected in dogs affected by diseases without clear-cut joint complications (Halliwell, 1978; Kaswan et al., 1983). In fact, various studies support the contention that in dogs, as in man, RF may occur

in any chronic process with constant antigen–antibody interaction (e.g. Fritz and Rose, 1980; Pope et al., 1981). Our own observations suggest the frequent occurrence of anti-gamma globulins (mostly non-RA-associated) in our dog population. In considering all these findings on the incidence of anti-dog IgG's, the argument can be made that a ready accessibility of this repertoire would incontestably open a whole area of research and experimentation towards the understanding of the etiopathogenesis of 'real' RF.

In this study we have evaluated the suitability of three methodologies for determining the true incidence of anti-IgG factors in a dog fluid population comprising various normal and diseased body fluids, the latter including 26 potential RF holders (i.e. from dogs with diagnosed RLD): latex fixation test, passive haemagglutination test and gel precipitation test. Two facts that should bear on the use of assays for future detection of dog RF have emerged from this study. First, canine versions of the RF assays, as recommended to date in human practice (i.e. RW and LFT), may grossly underestimate the true state of affairs. Second, canine RF assays employed in this investigation produced substantially differing results, when based on the total number of anti-IgG detected. The RW which, in human practice, is generally preferred for reliability, turned out to be the poorest method for the detection of RF in dogs, at least in our hands. However, it is of interest, for future application of the RW, that our results using the RW and a sensitised dog red cell technique, were identical (cf. Table 2). Consonant results on human sera are reported by Koritz et al. (1980). The use of the passive HA test instead of the RW should save time, since absorption with dog red cells and heat inactivation are not necessary. Furthermore, the coupling of aggregated IgG to dog red cells by chromic chloride is easily achieved and quite reproducible.

The latex test was found to detect substantially more sero-positive fluids than the RW-like tests. In evaluating this detection rate for anti-dog IgG antibody which is more than fourfold that detected by our RW, it is necessary to demonstrate that the increase is not due to artefacts caused by non-specificity of RF assay. As regards our LFT, the control experiments demonstrated that such artefacts are unlikely to occur in the assay described. There was no apparent non-specific binding of fluids in the fluid population examined. All LFT-positive samples were positive after retesting, whilst for some 70% of these positive specimens, specificity of the agglutination reaction was confirmed by inhibition studies. Our LFT thus unequivocally detected anti-IgG factors, most of which escaped detection by standard canine RW. Hence, in spite of general consensus among rheumatologists as regards the established validity of the RW, and rightly so for human practice, our results warn against its unconditional introduction as an equally valid tool for diagnosing RF in dogs.

In man, the availability of the very reliable but cumbersome and time consuming RW clearly facilitated the development of a latex assay, giving corresponding results (Singer, 1974). These latex particles with adsorbed human

IgG are now more commonly used as standard RF assay. For dogs, a comparable reference test is lacking. Consequently, no uniformity exists in sample handling and testing conditions for assessing RF in dog fluids. Whereas research in human practice was continuously aimed at further elucidating mechanisms responsible for the failure of RW tests and LFT to detect certain agglutinating RF (e.g. Hay et al., 1976; Hansson and Winblad, 1978; Giaever et al., 1984), research in dog practice did not seem to proceed further than establishing that RF activity might be lowered beyond detection because of sample handling (e.g. Wood et al., 1980).

The rational approach towards making progress in this area would then appear to be the re-addressing of all theoretical sources of failure at work in the RF assays employed. In the present situation these probably include both assay-related and sample-related impediments to assay reactivity. In a recent report evidence is presented to indicate that both sources might either positively or negatively influence the outcome of the RF assay by predetermining the result of the battle between indicator IgG and test fluid IgG for binding sites on RF (Giaever et al., 1984). In this battle for binding sites, the use of increased concentrations of aggregated indicator IgG in combination with simultaneous minimisation of native IgG aggregation would allow an increased RF detection rate, since aggregated IgG has a higher avidity for RF than does monomeric IgG. Thus, the present results would then imply that the higher detection rate as compared to that of the other RF assays, must reflect apparent better 'RF testing conditions' present in the GPT. One explanation for this intrinsic high capacity of the GPT to detect RF present at variable concentrations can be readily given, i.e. the use of aggregated indicator IgG at relatively high concentrations (in our GPT at 7 mg/ml) in a double diffusion test system. The concentration used thus guarantees an excess of aggregated indicator IgG at each diffusion level, to compete successfully with most of the endogenous IgG of the fluid population examined. (Consequently, the GPT also allows the detection of anti-IgG's present at very low concentrations ('irrelevant' RF).) In both the LFT and the HA assays much less aggregated indicator IgG is used and consequently these assays are more restricted in their RF detection capabilities than the GPT. By the same token, testing conditions allowing aggregation of native IgG (e.g. heat-inactivation) will incontestably influence the outcome of LFT and/or HA more negatively than that of GPT. This phenomenology has been extensively studied by Hansson and Winblad (1978). Our own findings (cf. Table 4) are similar to their RF studies, utilising improved testing conditions where substantially improved results were obtained by RW, but not by LFT. However, in our study, some improvement in LFT results was also observed. Of particular interest is that improvement of both the RW and LFT results was achieved on arthritic sera, i.e. potential RF holders. These results clearly indicate that to date standard canine RF–LFT and RW fail to

TABLE 4

Anti-IgG antibodies as detected by standard[a] RW/LFT and new[b] RW/LFT

Category of sera	No. tested	No. RW positive by		No. LFT positive by	
		Standard RW	New RW	Standard LFT	New LFT
Normal	20	0	6 (0)[c]	10	11 (8)
Arthritic	40	9	19 (9)	17	26 (15)
Non-arthritic	30	3	10 (3)	20	19 (16)

[a]As described in Materials and Methods.

[b]Complement inactivation and test procedure in brief: 0.2 ml of EDTA (0.02 *M*), pH 7 + 1 ml of serum for 2 h at room temperature; 0.07 g NaCl was then added to this mixture and left overnight at room temperature. Then the RW (or LFT) was performed using a solution of 0.01 *M* glycine in 0.9% NaCl, pH 7.1 as diluent in the titration. Standard RW (or LFT) was performed in parallel and results obtained by both methods compared. All sera were retested three times and the specificity of the reaction was confirmed by inhibition studies.

[c]Values in parentheses denote the number of sera also positive by standard RW (or LFT).

detect (relatively) many RF including some with possible arthropathogenic potential.

This study emphasises that immunological reactivity to IgG, which is an important criterion in helping make the diagnosis of RA, is easily induced in dogs. The proviso here apparently is the existence of a chronic condition, either with or without clinical expression of disease (cf. Introduction). Our findings also underline the fact that the mere presence of circulating anti-gamma globulins does not suffice to produce clinical expression of RA, presumably because of either medication used, or absence of other factors required. For dogs, these theoretically include genetic, hormonal, and almost certainly also RF-related factors (i.e. quantity and quality of circulating RF). Certainly, carefully selected experimental routine methodologies, allowing the quantitative detection of the RF repertoire present for comparative RF studies, are necessary if any progress is to be made in this complicated area. While no single, standardly used, assay in this study would, as yet, satisfactorily fulfill this criterion, the improvement of RW and LFT results by the mere use of a better complement-inactivating procedure is indicative of possible further improvements.

We believe that for future work along these lines, there would clearly be a need for a useful RF 'indicator' test. While the failure to indicate titres is a serious drawback to the GPT–RF assay, its unparalleled capacity to detect RF present is not contested. We therefore suggest the use of the GPT as RF 'indicator' test, on theoretical grounds also, to indicate the test fluid's potential to agglutinate IgG-coated particles. By modifying the variables shown to determine the value of an RF assay, it should be possible to standardise the LFT and/or RW to either fulfill or approximate the aforementioned assay criterion.

Finally, the use of optimized canine LFT/RW could undoubtedly help to overcome the now impeded full description of RF characteristics and reactivities because of the, as yet, low detection rate of canine RF. We intend to use them in collecting material relevant to our studies currently under way in a dog (and rat) model for human RA.

While this paper was awaiting publication, various studies have reported consonant results obtained with humans. We here cite Kazyumba et al. (WHO collaborative study, 1986) who, in the discussion on their findings and those of others, state: "It appears now that B cell precursors for Fc-specific anti-IgG antibody-producing cells exist with relative high frequency in normal individuals and that stimulations leading to a significant expression of the B cell repertoire would lead to the production of RF". Our contention that in dogs, chronicity of a condition provokes RF production is compatible with the reported human situation, again stressing the validity of the dog RA model.

ACKNOWLEDGEMENTS

The authors are indebted to Ms. Aletta de Beaufort for her secretarial assistance.

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