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RABBIT RHEUMATOID FACTORS AS IMMUNE COMPLEX DETECTORS IN MAMMALIAN SERA

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Rabbit rheumatoid factors (RFs) that were produced during long-term immunization of these animals with homologous aggregated IgG (AAIgG RF) as well as with bovine serum albumins (AIC RF) were shown to be capable of reacting with heterologous IgG aggregated by heat. This cross reaction was used as a basis for the detection of IgG immune complexes (IC) in man and animals. It was shown that some IgG IC in human pathological sera could be detected only by rabbit RFs. Namely, 7% of 80 sera inhibited the agglutinating activity of AAIgG RF and 1.2% of AIC RF exclusively. Human RFs, either monoclonal or polyclonal, were inefficient in detecting IC in these sera. Both rabbit RFs were, however, not able to detect IC in the sera of arthritic dogs or horses immunized by toxoid tetanus, which were IC positive in other tests. The results were discussed from aspects of conformational characteristics of Ig in IC.

Key words: Rabbit rheumatoid factors; immune complex detection.

INTRODUCTION

It is known that circulating immune complexes (IC) can stimulate the synthesis of auto-antiimmunoglobulin antibodies (rheumatoid factors-RF) specific for the Fc portion of autologous IgG altered through interaction with primary antigen. Because of its ability to react with IC, not only in vivo but also in vitro, the rheumatoid factor can be used for the detection of CIC under pathological conditions. One of the few tests utilizing RF for IC detection is based on the properties of RF to agglutinate polystyrene particles coated with human IgG (Latex). When such RF is mixed with a serum containing immune complexes, the IC combine with RF and neutralize its agglutinating activity. For this test, human RF (Luhruma et al. 1976) as well as rabbit IgM antibodies specific for native (Levinsky and Soothill 1977) and aggregated (Milošević-Jovčić 1979) human IgG were applied. However, different RFs may possess different specificity. That is why all IgG IC cannot be detected by one RFs, of which heterologous anti-Ig antibodies seem to be worthy of attention. Some of them were shown to be

capable to react with heterologous IgGs aggregated by heat. Since IgGs aggregated by heat are a convenient in vitro model of CIC, it might be possible to use this cross reaction as basis for the detection of IgG IC in man and animals.

In this work we searched for two categories of rabbit RFs as CIC detectors in man and some mammalian species. Their synthesis was induced by long-term immunization of animals with their own IgG previously isolated and aggregated by heat (AAIgG RF) and with bovine serum albumin. The latter is known as an immunogen which can form long-persistinng complexes with corresponding antibodies; these complexes can stimulate RF synthesis (AIC RF) (Milošević-Jovčić et. al. 1995).

MATERIAL AND METHODS

Serum samples

Human sera originated from healthy subjects as well as from patients with various systemic diseases. Animal sera were obtained from healthy horses, sheep, cattle, dogs, guinea pigs and nonimmunized rabbits.

Models of immune complexes

a) Aggregated IgG. The IgG fraction was isolated from normal mammalian sera by the Rivanol / ammonium sulphate procedure (Heide and Haupt, 1964) as well as by chromatography on DEAE Sephadex using a continuous gradient of phosphate buffer (pH 7.) of different molarity (0.01-0.3M) (Michaelsen, 1973). Isolated IgGs were exposed for 10-30 min (depending on the species) to a temperature of 63^0C.

b) IC prepared in vitro. Bovinne serum albumin (BSA)/anti-BSA and toxoid tetanus (TT)/anti-TT IC were prepared in 3x, 5x, 10x and 30x antigen excess (Hudson and Hay, 1980).

Rabbit rheumatoid factors (RF)

Rheumatoid factors were obtained by long term immunization of rabbits (Chinchilla) with homologous IgG aggregated by heat as well as with BSA (Milošević-Jovčić et al. 1995).

The titers of RFs were determined by agglutination of latex particles coated with human IgG (Singer and Plotz, 1956).

The reactivity of rabbit RFs with native and aggregated IgGs and with IC prepared in vitro was studied inan agglutination inhibition system by estimating the inhibitory activity of IgG and IC samples.

Detection of circulating IC

Circulating IC were detected by an RF agglutination inhibition method using rabbit RFs as IC detectors. Human Polyclonal (pRF) and monoclonal (mRF) RFs were used in the same system for comparison. Circulating IC were determined in 80 human pathological sera, in sera of dogs with arthritis and in sera of horses immunized by toxoid tetanus.

The level of IC was estimated using the standard curves constructed for both RFs after regression analysis (Milošević-Jovčić, 1984): quantities of 5-200 μg of aggregated IgGs were added to 1 mL of normal human serum and the

inhibition capacity of each sample towards both rabbit RFs was determined. IC levels were expressed as micrograms of aggregated IGG equivalents per milliliter (AggIgG μEq/mL).

RESULTS

Reactivity of rabbit RFs with aggregated IgG

Both types of RFs agglutinated latex particles coated with human IgG in relatively high titers (1:256 for AAIgG RF and 1:512 for AIC RF). Agglutination provoked by rabbit RFs could be inhibited by human, rabbit, ovine, bovine, equine and guinea pig IgG aggregated by heat, the inhibition being most intensive when molecules of homologous (rabbit) and human IgG were brought into contact with both RFs. Canine aggregated IgG as well as nantive IgG preparations of each species did not display inhibiting activity towards these RFs (table 1).

Table 1. Inhibition of rabbit RF agglutination by native (n) and aggregated (b) IgG of various mammalian species

Rabbit RFs	IgG – titers of inhibition													
	human		rabbit		ovine		bovine		eguine		canine		guinea pig	
	n	a	n	a	n	a	n	a	n	a	n	a	n	a
AAIgG RF	1:2	1:16	–	1:16	1:2	1:8	–	1:4	–	1:8	–	–	–	1:4
AIC RF	1:2	1:16	–	1:16	–	1:8	1:2	1:8	–	1:4	–	–	–	1:4

Reactivity of rabbit RFs with IC prepared in vitro

Homologous (BSA/anti-BSA) and heterologous (tetanus toxoid/human anti tetanus toxoid) immune complexes prepared in vitro also inhibited the agglutination of these RFs over the broad range of antigen (Ag)/antibody (Ab) ratios from a three to thirty fold excess of Ag. Homologous IC achieved the maximal inhibition in 10 times antigen excess, whereas heterologous IC maximally inhibited both RF s in approximately three times Ag excess.

The majority of normal human as well as animal serum samples did not inhibit rabbit RFs. Those that displayed inhibition acted in low titers (1:2) only.

Reactivity of rabbit RFs with IC formed in vivo

When both rabbit RFs were applied for CIC detection in 80 sera from patients suffering from various diseases, it was shown that 27,5% of the tested sera displayed an inhibiting activity when AAIgG was used as a CIC detector, and 18,7% of the sera inhibited the agglutinating activity of AIC RF (table 2). The inhibition titers were higher than those achieved by normal human sera going up to 1:64. AAIgG RF revealed increased values for CIC in 17 and AIC RF in 11 of the tested sera if the CIC level was expressed in micrograms of aggregated human IgG equivalents/ml. All values higher than 20 μg AIgG Eq/mL were considered as an increased level of CIC. Standard curves for this estimation were made separately for both rabbit RFs.

In 7% of tested sera CIC could be registered only by AAIgG RF and in 1,2% of tested sera this could be done only by AIC RF. No other RFs, either human

(polyclonal and monoclonal) or rabbit specific for human aggregated IgG, detected IC in these sera (table 3).

Table 2. Percent of investigated human sera which reacted with different RFs

RF		%
Human:	mRF	18.7
	pRF	32.5
Rabbit:	AAIgG RF	27.5
	AIC RF	18.7

Table 3. Percent of sera which reacted with one of the various RFs only

RF		%
Human:	mRF	1.2
	pRF	2.5
Rabbit:	AAIgG RF	7.0
	AIC RF	1.2

Arthritic canine sera and sera of horses immunized by toxoid tetanus did not inhibit rabbit RFs.

DISCUSSION

Circulating immune complexes are involved in the pathogenesis of many human and animal diseases. Their measurement in serum is considered to be useful in the assessment and monitoring of the pathological process. Rheumatoid factor (RF) has been used as a biological reagent for the detection of IgG IC of a smaller size. However, each RF may have unique specificity against particular gamma globulin conformational determinants and detect only one type of IC. The results of this work show that some IC can be detected only by heterologous RFs. Human sera which failed to react with homologous RFs (mRF and pRF) inhibited the agglutinating activity of rabbit RFs. In 7% of the investigated sera, IC could be detected only by AAIgG RF and in 1.2% only by AIC RF.

It is known that rabbit RF is capable of reacting with human IgGs (Milgrom and Witebsky, 1960), especially when they are aggregated or involved in AgAb complexes. The ability of rabbit RFs to detect IC which were unreactive with homologous RFs might be due to the IgG subclass involved in the complexes. It is possible that conformational changes of human IgG of a given subclass incorporated in IC might make these molecules very similar to altered rabbit IgG. The consequence could be that some human IC are not able to inhibit the activity of homologous RFs. In some way such a possibility was supported by a study (Masson et al. 1977) where AgAb complexes in human pregnancy were detected exclusively by rabbit RF which was induced by injecting in to animals autologous aggregated IgG. Human RF was ineffective in detecting those IC.

Differences in reactivity with IC have been registered not only between rabbit and human RFs but also between the two rabbit RFs. Although both AAIgG RF and AIC RF reacted strongly with human aggregated IgG1 subclass carrying

G1m(f) and "non a" allotypic markers, which suggests the presence of this isotype in IC, they differed in their capacity to detect IC in human sera. This indicates that conformational changes of Igs in IC, even though the Igs belong to the same isotype, may differ. It is possible that among RFs from some rabbits there is a population reactive not only with Fc but also with the Fab part of IgG in IC. Although anti-Fc and anti-Fab atibodies have generally been regarded as separate populations of anti-IgG antibodies, both in humans and in rabbits (Milgrom and Kano 1978, Hunt et al. 1990), there is evidence which suggests that among human RFs antibodies with dual specificity for both the Fc and Fab fragment exist (Hunt et al. 1990). If such RFs exist in the sera of some hyperimmunized rabbits, their reactivity with human IC may be due to an additional conformational complexity of antibody in IC.

Both rabbit RFs reacted with aggregated IgG of some other mammals. This indicates that they may be used for CIC detection in the sera of animals too. Unfortunately, sera of animals with diseases known to be associated with CIC were unavailable, except those of dogs with arthritis. Rabbit RFs, however, seem to be unsiuitable for CIC detection in canine sera since canine aggregated IgG does not inhibit agglutinantion caused by rabbit RFs. Moreover, IC in canine arthritic sera, the presece of which was registered by direct PEG test (unpublished data), could not be detected with any of the RFs used. One other type of RF obtained by immunizing rabbits with human aggregated IgG was also shown to be unreactive with canine IgG (Nikolić et al., 1983). It is possible that the size of IgG aggregates or immune complexes as well as the manner in which aggregation occurs may be responsible for their inability to react with RF (Oreskes and Mandel, 1984, Milošević-Jovčić et al., 1987). Heat aggregation or IC formation may in some IgG molecules block the reactive sites essential for RF binding. This, for instance, was shown to be the case with human IgG3 (Augener and Gray 1970, Capra and Kunkel 1970, Izui et al. 1994) and porcine IgG molecules (Metzger et al., 1975).

The fact that some human (or animal) IC an be detected by cross-reactive rabbit RFs raises the question of the characteristics of such IC, their biological activities and potential pathophysiological role.

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REUMATOIDNI FAKTORI KUNIĆA KAO DETEKTORI IMUNOKOMPLEKSA U SERUMIMA SISARA

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SADRŽAJ

Dugotrajnom imunizacijom kunića homologim IgG agregiranim toplotom i govedim serumskim albuminima indukovana je sinteza reumatoidnih faktora (RF) koji su ispoljili reaktivnost sa agregiranim IgG čoveka, ovce, krave, konja, zamorčeta. Kako agregirani IgG predstavljaju modele imunokompleksa in vitro, ova unakrsna reakcija je iskorišćena za otkrivanje i određivanje količine imunokompleksa u serumima bolesnih ljudi i nekih životinja. Pokazano je da se neki imunokompleksi u ljudskim serumima mogu otkriti samo kunićevim RF-ovima, a da istovremeno nisu mogli biti registrovani homologim, monoklonskim i poliklonskim, RF-ovima. Kunićevi RF-ovi nisu detektovali imunokomplekse u serumima pasa sa artritisom niti konja imunizovanih toksoidom tetanusa, a u čijim su serumima imunokompleksi dokazani drugim postupcima. Specifičnost komformacionih promena imunoglobulina u imunokompleksima je diskutovana kao osnov reaktivnosti imunokompleksa i razih reumatoidnih faktora.