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BRIEF COMMUNICATION

Binding of *in vivo* formed immune complexes by monoclonal mouse rheumatoid factors

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Summary. Mouse rheumatoid factors (RF) have previously been shown to bind immune complexes formed *in vitro* with greater affinity than monomeric IgG. The present report extends this observation and shows that this property of mouse RF is retained with immune complexes formed *in vivo*. This observation strengthens the idea that the interaction of RF with immune complexes could play a physiological role.

Recent studies have demonstrated that, in the mouse, isotype-specific RF are regularly produced during secondary immune responses against protein antigens (Coulie & Van Snick, 1983; Nemazee & Sato, 1983). The formation of immune complexes, which follows the injection of antigen under these conditions, could play a critical role in the development of these RF responses. It was, thus, of interest to determine if mouse RF were able to react with immune complexes formed *in vivo*. This investigation was greatly facilitated by the availability of a collection of monoclonal isotype-specific mouse RF (Van Snick, Stassin & de Lestré, 1983).

Monoclonal IgM RF were purified by euglobulin precipitation, gel filtration on Ultrogel AcA22 (LKB Produkter, Bromma, Sweden) and preparative electrophoresis in Pevikon (Serva, Heidelberg, Federal Republic of Germany). Their interaction with IgG was tested in a solid phase radioimmunoassay (RIA) using polyvinyl chloride flexible microtitre plates (Flow Laboratories Inc., Rockville, MD) coated by overnight incubation at room temperature with monoclonal IgM RF specific for IgG1 or IgG2a (3 μg/ml in 0·03 M NaCl, 0·02 M glycine pH 9·2). After washing in saline containing 0·01% Tween 20 (Technicon Chemicals, Orcq, Belgium), the plates were first incubated for 2 hr at 37° with the samples to be tested, followed by ^{125}I-labelled rabbit antibodies specific for mouse IgG1 or IgG2a. The radioactivity that was bound to wells coated with a monoclonal IgM devoid of RF activity was subtracted.

We first verified the capacity of insolubilized RF to preferentially bind complexed IgG. Immune complexes were formed *in vitro* in slight antibody excess (2·4-fold) by incubating 3 μg monoclonal IgG1 (A6204G12) or IgG2a (A6205C1) anti-DNP antibodies with 25 ng dinitrophenylated bovine serum albumin (DNP45-BSA) in 250 μl tris-buffered saline (TRIS 10 mM, NaN_3 10 mM, NaCl 130 mM, pH 7·5) supplemented with 5% fetal bovine serum for 2 hr at 37°. As shown in Table 1, insolubilized RF bound 100-fold more complexed than free IgG. Similar results were obtained with many different monoclonal RF (data not shown).

In order to find out whether RF also reacted with immune complexes formed *in vivo*, we tested the capacity of insolubilized monoclonal RF to detect the formation of antigen-antibody complexes in the serum of immune animals challenged with antigen. This approach was preferred over the conventional determination of putative immune complexes in lupus-prone animals because it avoids the bias introduced by

Abbreviations: BSA, bovine serum albumin; RF, rheumatoid factor; RIA, radioimmunoassay.

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Table 1. Binding of free and complexed anti-DNP antibodies to insolubilized RF*

Anti-DNP antibody	IgM anti-IgG1 RF	IgM anti-IgG2a RF
Free	200	100
Complexed	40800	20400

* Wells coated with monoclonal anti-IgG1 (1307A6) or anti-IgG2a (A8305H6) IgM RF were incubated as described with 2 μg/ml free or complexed monoclonal IgG1 or IgG2a anti-DNP antibodies, respectively. Results are expressed in c.p.m. (mean of triplicate measurements) after subtraction of non-specific bound radioactivity.

the hyper-γ-globulinaemia usually associated with this autoimmune condition.

Accordingly, BALB/c mice were immunized s.c. with 100 μg TNP48-KLH in complete Freund's adjuvant and boosted i.v. 4 months later with 50 μg of the same antigen. As verified by solid phase RIA just before the boost, the serum of these animals contained high levels of IgG1, and much lower levels of IgG2a anti-TNP antibodies (697±150 and 87±54 μg/ml, respectively). The binding of IgG1 complexes by insolubilized anti-IgG1 RF was tested in sera collected before and after the boost, and was found to dramatically increase immediately after injection of antigen (Fig. 1). In contrast, no change was detected after injection of the same amount of TNP48-KLH into unprimed mice.

With anti-IgG2a RF, a similar increase in binding was observed in the immunized group. However, the amount of IgG2a complexes detected under these conditions was considerably lower than that measured with anti-IgG1 RF, which reflected the predominance of IgG1 over IgG2a in the anti-TNP response of these animals (data not shown).

The results presented here formally prove the capacity of mouse RF to selectively bind immune complexes, even when they are formed *in vivo*. This observation supports the view that immune complexes could be involved in the induction of the RF synthesis, which occurs during the early stages of secondary immune responses. It also confirms and extends to an homologous system previous work showing that human RF can react *in vivo* with heterologous immune

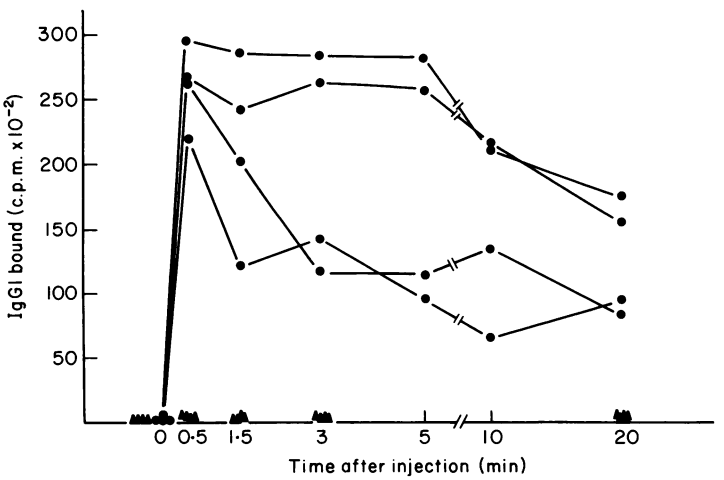

Figure 1. Binding of *in vivo* formed immune complexes by insolubilized RF. Sera were collected at different times after the i.v. injection of naive (▲) or immunized (●) BALB/C mice with 50 μg TNP48-KLH. Binding of IgG to insolubilized anti-IgG1 RF was measured by RIA, as described, in individual sera diluted 1/50 (mean of triplicate determinations).

complexes (Floyd & Tesar, 1979; Ford, 1983). Moreover, it suggests that the effects of RF on immune complex processing by macrophages (Van Snick *et al.*, 1978) and virus neutralization (Notkins, 1971), which have so far been determined chiefly *in vitro*, could also take place under physiological conditions.

Finally, our data demonstrate that it is possible to use mouse RF to assay immune complex levels. The availability of numerous isotype-specific monoclonal RF could prove extremely useful in this respect.

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