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The role of IgM rheumatoid factor in experimental immune vasculitis

M. FLOYD & J. T. TESAR *Departments of Medicine, Northwestern University Medical School and Veterans Administration Lakeside Hospital, Chicago, Illinois, USA*

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SUMMARY

The effect of IgM rheumatoid factor (RF) on reversepassive cutaneous Arthus reaction in rats was studied. The RF was obtained from the serum cryoglobulin of a patient with symptoms of purpura, arthralgia and digital gangrene. The cryoglobulin was of IgG–IgM type and when given i.v. it induced a prompt hypocomplementaemia in experimental animals. The purified RF also induced low serum complement levels when injected i.v. along with complexes of non-complement-fixing, aggregated IgG. A reverse passive Arthus reaction was induced by intradermal injection of IgG anti-bovine serum albumin (BSA), followed by an i.v. dose of antigen (Ag). The cutaneous inflammatory reaction was aggravated by simultaneous administration of IgM RF intradermally, but not by IgM without antibody (Ab) properties. Intradermal injection of low concentrations of non-complement-fixing IgG anti-BSA, along with normal human IgM, followed by i.v. injection of BSA, resulted in a complete lack of cutaneous inflammation. At higher Ab concentrations there was only a mild inflammation. However, when IgM RF was substituted for normal IgM and injected with non-complement-fixing anti-BSA, an effective reverse passive cutaneous Arthus reaction and vasculitis was induced. The inflammatory response was greatly suppressed by decomplementation of animals by cobra venom factor. This study provides evidence favouring an inflammatory, complement-dependent role for RF in vasculitis.

INTRODUCTION

Systemic manifestations of rheumatoid disease such as vasculitis, neuropathy, pulmonary fibrosis and episcleritis are frequently associated with high serum titres of IgM RF (Gordon, Stein & Broder, 1973; Epstein & Engleman, 1959). The reasons underlying the association between RF and the complicated clinical course are not clearly understood. Some studies suggest a protective effect (Schmid & Roitt, 1966; Davis & Bollet, 1964), while other investigations indicate an active role for RF in the development of synovial inflammation (Zvaifler, 1973) and of rheumatoid vasculitis (Pernis, Ballabio & Chiappino, 1963).

Since the information about the *in vivo* role of RF is ambiguous, this study was designed to determine whether IgM RF can induce or aggravate cutaneous inflammation and vasculitis in an experimental animal model. The use of non-complement-fixing IgG Ab and purified RF in a model of cutaneous reverse passive Arthus reaction (RPCA) permitted the evaluation and quantification of the effect of RF in the induction of vascular inflammation.

MATERIALS AND METHODS

Patient. The source of IgM RF was the serum of a patient with symptoms of purpura, arthralgia, hepatomegaly, intermittent claudication and digital gangrene. The serum had low levels of complement, high titres of RF (1:25,000) and large quantities of IgG–IgM type mixed cryoglobulin (MCG).

Correspondence: Dr J. T. Tesar, Rheumatology Service, Walter Reed Medical Center, Washington, D.C. 20012, USA.

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Mixed cryoglobulin. The plasma containing cryoglobulin was recalcified and clotted at 37°C for 120 min and the fibrin clot was removed by filtration through a sterile gauze. A cryoglobulin precipitate was formed by incubation at 4°C for 48 hr. It was sedimented by centrifugation at 1500 *g* for 10 min. This was done five times. The purified cryoglobulin was analysed by immunodiffusion and immunoelectrophoresis (IE) using monospecific anti-IgG, IgA, IgM, C3, C4, C3PA (factor B), C1q (Hoechst, Sommerville, New Jersey.) and polyvalent horse anti-human serum (Hyland Travenol Laboratories, Los Angeles, California). C1q and C3 were found in the cryoglobulin only after the first wash. After five washes the cryoglobulin consisted entirely of IgM and IgG components identified by immunodiffusion.

IgM RF. 30–40 mg of the purified cryoglobulin in 1·0 ml was added to an equal volume of 0·2 M glycine HCl pH 2·8 buffer, then mixed and incubated at 37°C for 20–30 min. The insoluble pellet was discarded. The resultant supernatant protein solution was adjusted to 10% sucrose and applied to a 15–25% (w/v) discontinuous acid sucrose gradient made in 0·15 M glycine NCl pH 2·8 buffer. 30 ml polyallomer tubes were used in an International Equipment Company preparative ultracentrifuge (Model L) with SB110 rotor. This was centrifuged at 24,000 rev/min (70,000 *g*) for 18 hr at 4°C. The IgM fraction was obtained from the 16–18% sucrose layer by puncture of bottom of tubes and drop-wise collection of 20–25 1·0 ml aliquots. (Fig. 1) Each preparative run was accompanied by a tube of marker proteins. For this purpose we used ^{125}I IgM from a patient with Waldenström's macroglobulinaemia mixed with ^{131}I deaggregated pure human IgG. The distribution of radioisotope at the completion of each run was determined in 1·0 ml samples obtained by bottom puncture and counting in a well-type gamma counter (300 gamma counter, Searle, Chicago, Illinois). After extensive dialysis against phosphate buffered saline (PBS) at 4°C, fractions containing only IgM by double agar diffusion were pooled and concentrated by positive pressure in an Amicon cell, Model 202, using a UM10 membrane (Amicon Corp, Lexington, Massachusetts). The resulting solution was deaggregated by ultracentrifugation at 100,000 *g* for 60 min in a Spinco Beckman Model L ultracentrifuge and the upper half of the supernatant containing IgM RF was stored in aliquots at −70°C. This reagent was analysed for contaminant proteins by IE and double diffusion in agar. The only contaminant was IgG which was present in less than 0·1 mg/ml. The IgM concentration was between 4–5 mg/ml.

Human IgG. This was obtained from plasma Cohn fraction II (Pentex, Kankakee, Illinois) by ion exchange chromatography with Sephadex DEAE A50 (Sober *et al.*, 1965). The purified fraction contained only IgG when examined by IE and immunodiffusion with monospecific rabbit antisera to IgG and normal human serum.

Non-complement fixing IgG aggregates. Reduced alkylated and aggregated human IgG (MEAGG) was prepared by the method of Widerman, Miescher & Franklin, 1963). After extensive dialysis against PBS, the IgG (10 mg/ml) was aggregated by heating at 63°C for 30 min and stored at −70°C.

Normal human IgM (NIgM). Human IgM without anti-globulin properties was obtained from the serum of a patient with Waldenström's macroglobulinaemia. Serum was dialysed in the cold against 0·005 M phosphate buffer pH 7·1. The precipitate was dissolved in PBS and applied to a Sephadex G200 superfine column (125 × 2·5 cm) equilibrated with this buffer.

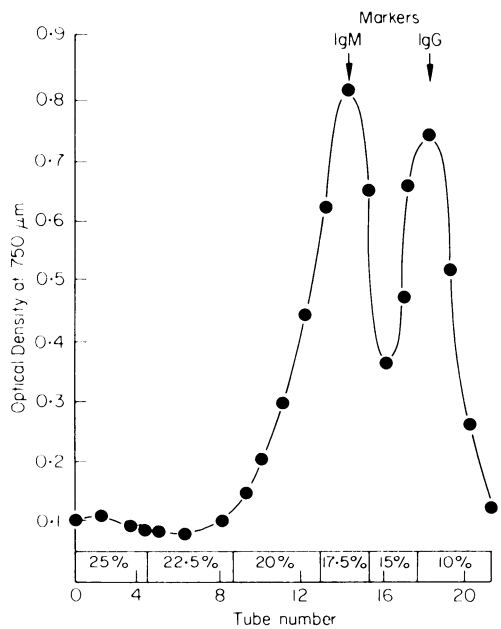

FIG. 1. The separation of two components of MCG by ultracentrifugation in sucrose pH 2·8. The IgM component was found in 17·5% sucrose layer and the IgG was retained in the 10–15% fractions after 18 hr centrifugation at 24,000 rev/min (70,000 *g*).

The resultant IgM peak was concentrated by ultrafiltration and separated from minor contaminants by DEAE A50 Sephadex chromatography using a Tris glycine HCl buffer pH 8·0 with an ionic gradient from 0·005 M to 0·4 M. The product was found to be homogenous IgM on IE and polyacrylamide gell electrophoresis (Boesken, Kopf & Schollmeyer, 1973; Scheidegger, 1955).

Quantification of IgG and other proteins. The concentration of human IgM and IgG and rabbit IgG was estimated by radial immunodiffusion using commercial plates for the human proteins and immunoplates prepared in this laboratory for the rabbit IgG (Mancini, Carbonara & Heremans, 1975). Determinations of other proteins were done by the method of Lowry *et al.* (1951), using freeze-dried human IgG or BSA as standards.

Antigens. Crystalline (re-crystallized three times) BSA was obtained from Pentex Co., Kankakee, Illinois. Pure rat serum albumin was prepared by the methods of Korner & Debro (1956). The rat serum albumin did not cross-react with BSA when tested in a passive haemagglutinating system with anti-BSA (ABSA).

Animals and antisera. NZW rabbits weighing 2·5 kg were immunized intramuscularly with 5·0 mg of BSA in FCA at monthly intervals for 6 months. They were bled 10 days after a final boosting injection of 2·0 mg BSA in saline. Female Wistar rats weighing 200 g were used for *in vivo* studies.

Purified ABSA. Rabbit ABSA was obtained from the immune rabbit serum using a solid phase immunoabsorbent. The serum was incubated at 56°C for 30 min and repeatedly absorbed with SRBC to remove heterophile Ab. BSA Ag was covalently coupled to Sepharose 2B by the method of Cuatrecas (1970). The immune serum was stirred with the immobilized BSA for 30 min at 37°C and then for 18 hr at 4°C. The immunoabsorbent was then washed in a column with PBS until the effluents gave an optical density of less than 0·005 at 280 mμ. The Ab was eluted with 0·2 M glycine HCl buffer pH 2·8 and immediately and extensively dialysed against PBS at 4°C. The product was entirely IgG when it was examined by IE, using goat anti-whole rabbit serum and goat anti-rabbit IgG (Scheidegger, 1955). The product was concentrated, deaggregated by ultracentrifugation for 60 min at 100,000 *g* and stored at −70°C.

BSA–ABSA immune complexes. ABSA (4·0 mg/ml) and dilutions of BSA (4·0–0·02 mg/ml) were mixed in equal volumes at room temperature. The precipitate obtained from the tube at equivalence was washed once and stored at −20°C.

Carbamylated rabbit ABSA (CAB). Preliminary *in vivo* experiments established that 2-ME treated and alkylated ABSA had non-specific irritating properties when injected intradermally in rats. To avoid this, the ABSA was carbamylated by the method of Chen, Grossberg & Pressman, (1962). The final product had an unchanged Ab titre and was devoid of complement-fixing and skin irritant properties.

Determination of RF reactivity with ABSA and CAB. Latex particles 0·8 μ size (Difco Co., Detroit, Michigan), were coated with either ABSA or CAB. Both types of sensitized particles were strongly agglutinated by a purified IgM RF solution.

Radiolabelling of proteins. The method of McConahey & Dixon (1976) was used.

Double agar diffusion. Precipitation of Ag by Ab in agar was performed by the method of Ouchterlony & Nilsson (1973).

Complement fixation. The micro-CFT of Wasserman & Levin (1960) was used with a modification of the incubation volume to 5·0 ml. In experiments were MCG and RF were used, the first stage incubation proceeded at 0°C for 30 min, followed by incubation at 37°C for 30 min to permit an efficient activation of the entire complement sequence.

Haemolytic complement determination. Serum complement was measured in some *in vivo* studies using the method of Mayer (1961), as modified and described by Tesar & Schmid, (1970). The results are expressed as a percentage of normal complement levels in controls.

In vivo inhibition of rat complement. Decomplementing of experimental animals was achieved by i.p. administration of 25 μ of cobra venom factor per 100 g body weight in three divided, successive doses. The remaining haemolytic complement was approximately 5% of the initial normal complement level.

Induction of RPCA. Initially, it was necessary to determine whether RPCA lesions could be accurately quantified. This was achieved by using a modification of the radioisotope method of Udaka, Takeuchi & Morat (1970). Fifty rats were shaved on both sides, 24 hr before the experiments. They were divided into five groups of ten (Fig. 2). Animals in each group received an intradermal injection in 50 μl volume of either 10 μg, 25 μg, 50 μg, 75 μg, or 110 μg of ABSA. The control sites consisted of injections of 50 μl of PBS and deaggregated normal rabbit IgG in quantities identical to ABSA. After 30 min, 10 mg of BSA per 100 g weight was administered i.v. along with 20 million ct/min of ^{131}I-labelled rat serum albumin and 5·0 mg Evans' blue to mark the area of injury. 40 min later, the animals were killed for quantification of vascular permeability (VP) of RPCA, since at that time the best Ab dose response effect was observed. A uniform skin piece of 15 mm diameter containing the RPCA lesion was excised for this purpose.

Calculation of VP. The excised skin pieces were counted in a Searle 300 gamma counter. A value for cutaneous VP was derived by correcting the ct/min in each excised skin piece to 100 mg weight and then dividing it by a simultaneously determined count in 50 μl of serum. The derived value multiplied × 100 was regarded as a convenient measure of VP.

Using this method of quantification it was found that there was a reproducible and direct relationship between the intradermal Ab concentration and the ensuing local vascular injury of RPCA (Fig. 2).

Induction of RPCA with IgM RF and (IgG) ABSA. A total of eight rats of uniform weight, divided into two groups were used. The animals were shaved on both sides 24 hr before the experiment.

Each animal was then injected intradermally on its side at two comparable sites with 30 μg of IgG ABSA mixed with 106 μg of NIgM (control sites) and on the corresponding opposite site with a mixture of 30 μg of (IgG) ABSA and 106 μg IgM RF. The molar ratios of IgG:IgM in both instances were 1·6:1. Other bilateral cutaneous sites received 30 μg ABSA and RF in lesser quantities (molar ratios of (IgG) ABSA:IgM RF = 16:1, 8:1 and 2·6:1 (see Fig. 6). 30 min later, the

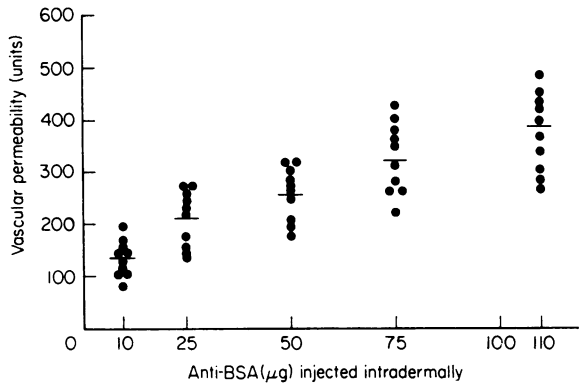

FIG. 2. Reverse passive cutaneous Arthus reaction. The relationship between intradermal anti-bovine serum albumin and local vascular permeability is shown. Ten animals in each group received intradermal injections of either 10 μg. 25 μg, 50 μg, 75 μg or 110 μg of Ab, followed 30 min later by i.v. injection of 10 mg BSA per 100 g weight. Control sites received equal quantities of rabbit Ig (deaggregated). The resulting VP was determined 40 min later. VP of control sites were negligible and are not shown.

first group of animals (Column 0) received i.v. only the Evans blue and ^{125}I rat serum albumin tracers. At the same time the second group (Column 5) received 5·0 mg BSA 100 g weight i.v. along with the tracers. The cutaneous VP was determined in both groups 40 min later, as described.

Induction of RPCA with CAB and RF. Eight rats were used and each was injected in three sites on each side. The first site received 10 μg CAB mixed with 22 μg NIgM; the second, 20 μg CAB with 44 μg NIgM; the third, 30 μg CAB with 66 μg NIgM. On the opposite side, the first site received 10 μg CAB mixed with 22 μg IgM RF; the second, 20 μg CAB with 44 μg IgM RF; the third, 30 μg CAB with 66 μg IgM RF. The molar ratios of IgG:IgM were maintained at 2·6:1.

Three additional control sites on each rat were injected. The first site received 30 μg CAB; the second, 66 μg RF; the third, 66 μg NIgM.

The animals were challenged 30 min later with i.v. BSA (10 mg per 100 g body weight). The VP of injected sites was determined 40 min later.

Determination of cutaneous reactivity to mixed cryoglobulins (MCG). Six rats were injected in five intradermal sites. Each site received a different substance, either PBS, RF, normal IgG and IgM, MCG or BSA-ABSA in 50 μl vol. per site. Radioactive tracer and 5·0 mg Evans' blue was injected i.v.

Another group of six rats was injected identically except that the injected skin sites were cooled for 30 min with ice cubes. Forty minutes later, animals in both groups were killed and the VP of the lesions was measured.

RESULTS

In vitro complement fixation by MCG (Fig. 3)

The purified MCG was shown to fix rat serum complement *in vitro*. The complement fixation was largely determined by the temperature of the initial incubation in the bithermic procedure. 22 μg of MCG consumed from 5% at 37°C to 57% of complement at 20°C initial temperature. Similar temperature response curves were obtained with greater concentrations of MCG bithermally incubated with rat complement.

Complement fixation by MCG complexes in vivo (Fig. 4)

It was observed that 2·0 mg MCG per 100 g body weight given i.v. to rats effected a more prolonged reduction in serum complement than the same dose of heat-aggregated IgG–IgM complex. The initial reduction in complement activity to 60–70% of normal was comparable in the two groups of animals.

Complement fixation by RF complexes in vivo (Fig. 5)

Experiments further demonstrated that *in vivo* IgM RF is capable of complement fixation when reacted with non-complement-fixing human IgG aggregates. 1·0 mg IgE RF per 100 g body weight given i.v. to rats was inert, whilst its administration following 1·0 mg of MEAGG induced a prompt and sustained hypocomplementaemia.

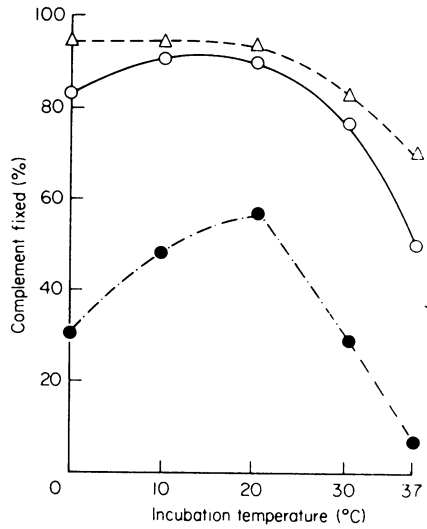

FIG. 3. The influence of first stage incubation temperature *in vitro* on rat complement fixation by MCG. Each sample was incubated for 30 min at a designated temperature, followed by incubation at 37°C for an additional 30 min. The largest quantity of complement was consumed at 20°C. (△) 72 μg MCG; (○) 55 μg MCG; (●) 22 μg MCG.

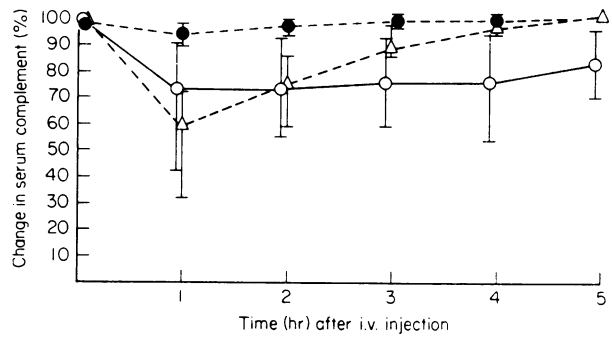

FIG. 4. Sequential changes in serum haemolytic complement after injection of MCG and other immune reactants. Each group of five rats received i.v. either, 2·0 mg MCG (○) 2·0 mg heat-aggregated IgG–IgM (△) or, 2·0 mg per 100 g weight (deaggregated) IgG–IgM (●).

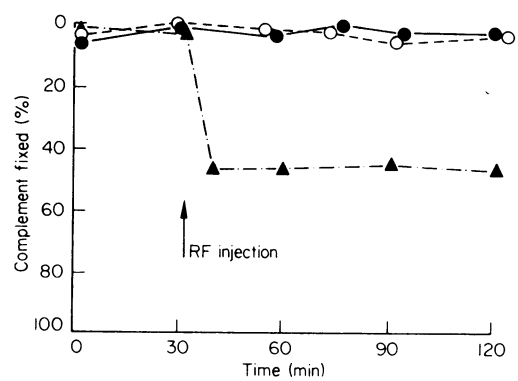

FIG. 5. The *in vivo* complement-fixing properties of IgM RF. Initially (0 min), two animals received 1·0 mg of MEAGG i.v. in 0·5 ml PBS. 30 min later, 1·0 mg IgM RF was given i.v. to one rat (▲), while PBS was given to the other (○). A third rat received IgM RF alone by the i.v. route (●).

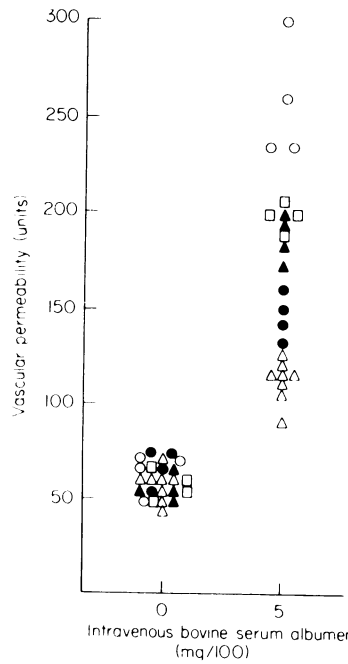

FIG. 6. Vascular permeability of skin sites injected with 30 μg ABSA mixed with NIgM or RF IgM. The ABSA was pre-incubated with NIgM or RF IgM at IgG:IgM molar ratios of 1·6:1, (△) (control). RF IgM was incubated at IgG:IgM ratios of 16:1 (●) 8:1, (▲) 2·6:1 ( ) and 1·6:1 ( ). ABSA was kept constant at 30 μg. The VP data were obtained from four control animals which received no antigen (Column 0) and four test animals (Column 5) which received 5·0 mg BSA i.v. after both groups had been injected intradermally with the indicated reagents. The most marked increase in cutaneous VP induced by RF was obtained at a molar ratio of IgG ABSA:IgM RF of 1·6:1.

Induction of RPCA by ABSA and effect of IgM RF (Fig. 6)

The effect of different quantities of IgM RF and its ratio to ABSA in the model of RPCA was investigated. In preliminary experiments it was established that with a uniform intradermal dose of 30 μg ABSA and 106 μg NIgM (IgG:IgM molar ratios 1·6:1) and increasing doses of BSA given i.v., the greatest degree of inflammation (80–135 VP units) was achieved in the group given 5·0 mg BSA per 100 g weight i.v. Measurable augmentation of inflammatory reaction by RF was achieved in a group of animals given 5·0 mg BSA per 100 g weight with the largest quantities of RF (IgG–ABSA:IgM RF = 1·6 or 2·6:1 molar ratio).

RPCA induced by CAB and RF (Fig. 7)

The role of RF in induction of increased cutaneous VP was investigated with the help of anti-BSA (IgG) Ab rendered non-complement-fixing by carbamylation. Such Ab by itself had no, or only a slight, inflammation-inducing property in RPCA. The group of animals which received 10 and 20 μg CAB and RF intradermally had the most pronounced cutaneous inflammation. The differences in inflammation in animals that received 30 μg CAB and RF intradermally were less marked compared to control sites.

Effect of cobra venom factor on RPCA induced CAB and RF (Fig. 8)

Four rats in each group (Column 10, 20 and 30) were pre-treated by cobra venom factor in order to determine the effect of decomplementation on RPCA, and were then injected with CAB and RF, and control sites with CAB and NIgM. The pre-treated group developed cutaneous lesions which were substantially reduced in size and VP when compared with the animals similarly treated but not receiving cobra venom factor.

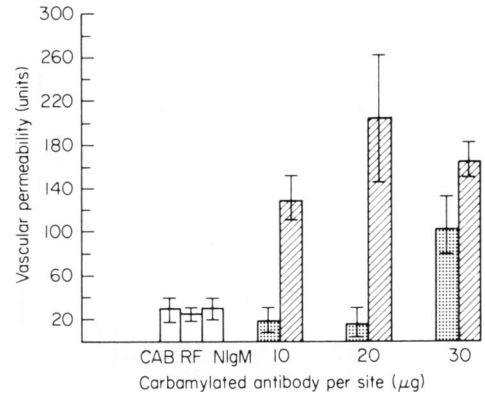

FIG. 7. Reverse passive cutaneous Arthus reaction. Comparison of VP changes induced by intradermal injection of CAB mixed with IgM RF (▨) with that of CAB mixed with NIgM (▦) followed by i.v. BSA challenge. The molar ratios of IgG:IgM were 2·6:1. RF induced a substantial increase in VP of RPCA at all doses used. Control sites (□) received separately CAB (30 μg); RF (66 μg) and NIgM (66 μg).

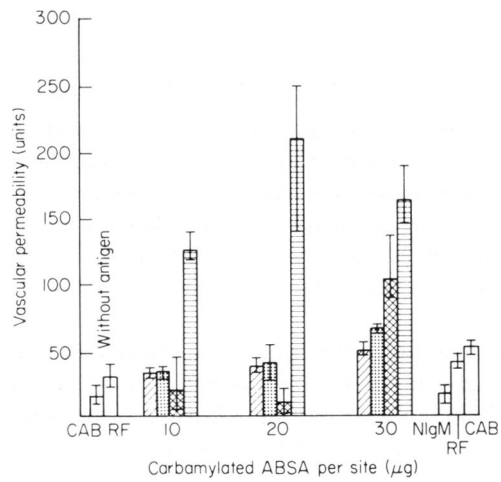

FIG. 8. Reduction of VP following cobra venom treatment of rats. Experiment performed as given for Fig. 7. In addition four rats were decomplemented with cobra venom factor and then received the same reactants as in Fig. 7. Four control rats (□) received only CAB (30 μg) and RF (66 μg) in separate skin sites. (▨) CAB pre-incubated with NIgM in cobra venom rats; (▦) CAB pre-incubated with RF IgM in cobra venom rats; (▩) CAB pre-incubated with NIgM in normal rats; and (▤) CAB pre-incubated with RF IgM in normal rats.

Intradermal effects of MCG and preformed immune complexes (Fig. 9)

Despite the demonstration complement-fixing properties of MCG and its IgM RF component after i.v. injection, the injurious effects of intradermal injection of MCG in the experimental animals was very slight. The acute inflammatory responses as measured by VP changes induced by RF alone (Column B), deaggregated IgG–IgM (Column C) and MCG (Column D) did not differ significantly from those induced by PBS (Column A). In contrast, the vascular injury inflicted by an equal concentration of stored rabbit immune complexes (Column E) was consistently severe.

These studies failed to show any significant difference between intradermal sites maintained at ambient temperature and those cooled with ice for 30 min and subsequently warmed for 30 min to body temperature.

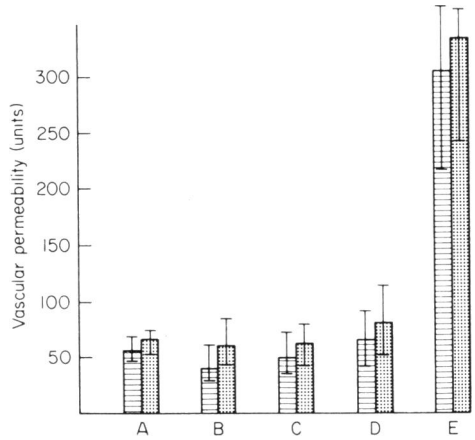

FIG. 9. Effect of skin cooling on VP induced by MCG and other immune reactants. Each of six rats received intradermal injections of (A) PBS, (B) 1·0 mg of deaggregated RF, (C) 1·0 mg deaggregated human IgG–IgM, (D) 1·0 mg MCG and (E) 1·0 mg BSA–ABSA complex in separate sites. Skin sites were cooled by application of ice cubes. (▩) The control animals were injected similarly but remained at ambient temperatures (▤). There was only a marginal increase of VP by MCG at both temperatures.

DISCUSSION

The experimental studies described in this report provide further and direct evidence for the role of IgM RF and its complexes in the mediation of increased VP and cutaneous vasculitis. The MCG used for isolation of RF had properties similar to cryoglobulins commonly found in rheumatoid vasculitis (Weissman & Zvaifler, 1975) and in essential mixed cryoglobulinaemia (Meltzer & Franklin, 1966). The isolated RF reacted with both human and rabbit IgG and had a quantitatively similar *in vitro* complement-fixing capacity to other antiglobulins found in rheumatoid arthritis (Tesar & Schmid, 1970; 1973).

When RF was injected i.v. along with aggregates of reduced and alkylated IgG, it resulted in prompt hypocomplementaemia in spite of the lack of anti-complementarity of each component by itself. By lowering complement levels, the antiglobulin reproduced a commonly associated manifestation of rheumatoid vasculitis (Mongan *et al.*, 1969) and IgG–IgM type of mixed cryoglobulinaemia (Riethmueller *et al.*, 1966).

When the antiglobulin was injected intradermally, its inflammation and specifically, its vasculitis-promoting effects were best demonstrated in a model of RPCA where the RF was complexed with non-complement-fixing (carbamylated) IgG Ab. RF also clearly potentiated the RPCA induced by untreated IgG Ab. The cutaneous inflammatory reaction was suppressed by the decomplementation of animals. This observation supports the view that complement is required for the promotion of inflammation by RF. Small or intermediate size IgG complexes do occur in the circulation of patients with RA, especially when associated with systemic complications. Our previously published data and the present results suggest that such complexes can interact both *in vitro* and *in vivo* with IgM RF and result in complement fixation (Tesar & Schmid, 1970).

Rather surprisingly, the MCG from which the RF was isolated had only minimal inflammatory effect when injected intradermally, in spite of its capacity to activate complement *in vitro* and in the circulation. The explanation of this deficiency is not immediately apparent but may relate to the rapid dissociation of the cryoglobulin complexes at tissue temperature into monomeric IgM and IgG.

VP associated with RPCA was measured 40 min after the injection of Ag, rather than 4–6 hr later as has been done in other studies. Our preliminary experiments established that at the earlier time increasing doses of Ab induced the most marked increments in inflammatory responses. This approach was supported by other studies showing that inflammatory phenomena characteristic of RPCA are

already developed shortly after the injection of Ag (Weber *et al.*, 1974; Udaka, 1971). In addition, the signs of cutaneous inflammation induced by the RF complexes in RPCA persisted for at least 6 hr and showed a histological pattern consistent with RPCA.

Several experimental studies have also implicated IgM RF in vascular damage, each in a different organ system or tissue. Baum, Stastny & Ziff (1964) have shown that RF and immune complexes injected into the mesenteric arteries of rats induced thrombosis and haemorrhage. McCormick *et al.* (1969) observed a potentiation of nephrotoxic nephritis following the i.v. injection of RF-containing sera. More recently, De Horatius & Williams (1972) induced focal pulmonary haemorrhagic infarcts and vasculitis by parenteral injection of RF into animals pre-treated by i.v. injection of Freund's complete adjuvant. Some additional support for the conclusions reached in this investigation can be found in the study of Fritz & Husberg (1969). These authors have induced a direct Arthus reaction in rabbits by the intradermal injection of rabbit IgG of the allotype to which they were previously immunized. It is known that some of the human RFs are also ABS against allotypic (Gm) IgG markers. Results of these studies, considered together, provide strong support for the inflammatory role of IgM RF in experimental vascular inflammation and, by inference, in rheumatoid vasculitis.

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