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Immune elimination and enhanced antibody responses: functions of circulating IgA

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Summary. Passively transferred MOPC 315 IgA myeloma protein accelerated the elimination of DNP-ovalbumin, to which the myeloma protein bound with antibody-like affinity, from the circulation and increased the subsequent antibody response to hapten and to carrier, when injected with or without adjuvant.

INTRODUCTION

Secreted IgA antibody has protective anti-microbial and antigen-handling activity (Fubara & Freter, 1973; Svennerholm, Lange & Homgren, 1978; Stokes, Soothill & Turner, 1975). IgA is also present in serum but there its function is little understood. It has been suggested that IgA–antigen complexes may be responsible for the development and maintenance of orally induced tolerance (Andre, Heremans, Vaerman & Cambiaso, 1975) but this assumes that it has effects on the handling of circulating antigens, which have not been reported. We have therefore studied the effects of passively transferred IgA on the elimination of antigen and on the subsequent antibody response to antigen, using the MOPC 315 IgA myeloma protein which has antibody-like affinity for DNP (Eisen, Simms & Potter, 1968).

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MATERIALS AND METHODS

BALB/c and CAF inbred strains of mice were maintained at the Institute of Child Health. Groups of 8–12 week old mice were matched for age and sex. Ovalbumin (grade 3) was obtained from Sigma Ltd (London) and conjugated with DNP by a modification of Porter's method (Porter, 1950) to achieve an average of four DNP groups per ovalbumin molecule. Monomeric DNP-ovalbumin was isolated by column chromatography on Sephadex G-200 (3·2 × 40 cm) and labelled with ^{125}I by a chloramine-T method (Hunter & Greenwood, 1962); it was monomeric on gel filtration (Sephadex G75; 1 × 12 cm).

BALB/c mice were bled 21 days after injection with 10^8 MOPC 315 tumour cells; their pooled serum contained ten times more IgA than normal mouse serum (measured by radial immunodiffusion). Anti-DNP activity in the pool was assayed by immunoprecipitation; briefly, 100 μl of pooled serum was incubated with monomeric DNP_4-ovalbumin (0–500 μg) for 7 days at 4°. One millilitre of the pool bound 250 μg of DNP_4-ovalbumin. An equivalence ratio of 3 moles of monomeric antibody per mole of antigen, calculated from this and assumption of normal mouse serum IgA concentration of 260 μg/ml provided a basis for relative doses for *in vivo* studies.

Two methods for measuring haemagglutinating antibody on microtitre plates (Titertek Flow) were used. For the adjuvantized response, direct agglutination of 50 μl of 1% sheep red blood cells (SRBC) coated with DNP (Hudson & Hay, 1976) or with ovalbumin

(chromic chloride method of Poston 1974), by doubling dilutions of 50 μl of serum, was used (end point, complete agglutination, no button). In some experiments, 50 μl of 0·1 M, 2-mercaptoethanol was added.

Response to non-adjuvantized antigen was measured by the antibody-enhanced haemagglutination assay (AEHA) (Coombs, Hunter, Jonas, Bennich, Johansson & Panzani, 1968) modified for use in microtitre plates. SRBC coated with DNP or with ovalbumin were incubated with 50 μl, of serially diluted serum at room temperature for 1 h. No agglutination was observed at this stage. Cells were then washed (×3) and incubated with 50 μl of rabbit anti-mouse IgG (1:500) for an hour before reading.

RESULTS

Antigen elimination by MOPC 315 IgA

Groups of eight CAF mice, receiving 0·2% potassium iodide in their drinking water, were injected in the tail vein with 50 μl of 1 in 50 or 1 in 500 MOPC 315 serum, or 1 in 50 normal mouse serum, diluted in saline. Thirty minutes later, 300 ng of monomeric DNP_4-^{125}I-ovalbumin in 50 μl ($1{\cdot}4 \times 10^6$ c.p.m.) was injected via the same route (equivalence and ten-fold antigen excess). The mice were bled from the retro-orbital plexus at 3 min, then hourly intervals for 5 h and radioactivity in the blood counted in a γ-counter (Pannax). In one experiment, trichloroaceic acid (TCA)-precipitated radioactivity was measured 1 h after antigen injection.

Antigen elimination (Fig. 1) was unusual in that the peak concentration was not obtained until 1 h (observed repeatedly for ovalbumin in mice but unexplained). The peak radioactivity was TCA precipitable (85–95%) and intact ovalbumin (gel filtration) so elimination was calculated after 1 h. All values in MOPC 315 serum treated groups were lower than in the controls, significantly so ($P < 0{\cdot}02$) except for the high dose group at 1 and 2 h; for the first 2 h the values from the antigen excess group were significantly lower ($P < 0{\cdot}02$) than those from the group at equivalence.

Effect of MOPC 315 protein on antibody response

Groups of eight BALB/c mice were injected intraperitoneally with 0·2 ml of MOPC 315 serum (1/5 or 1/50 in saline) or 1/50 normal mouse serum, followed 6 h later with 1·3 mg of DNP-ovalbumin in saline (i.e. 100- and 1000-fold antigen excess). This was repeated 7 days later and the animals were bled out 14 days later. IgG antibody titres (AEHA method) to DNP and ovalbumin were higher in the MOPC-315 treated mice than in the controls (1/50 mouse serum), significantly so to DNP in the 1/50 group (1000-fold antigen excess)

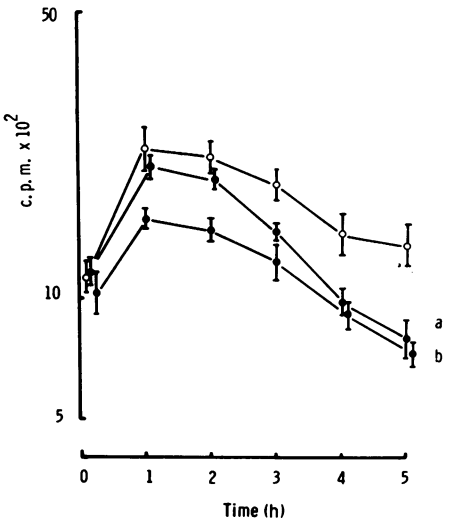

Figure 1. Radioactivity in 20 μl blood of mice after 300 ng of ^{125}I-labelled DNP-ovalbumin monomer i.v. at time 0, in mice given 50 μl of either MOPC 315 IgA myeloma serum (•) (a, 1 μl; b, 2 μl), or 1 μl normal mouse serum (○) i.v. 30 min before. Log mean values from groups of eight mice ± SEM.

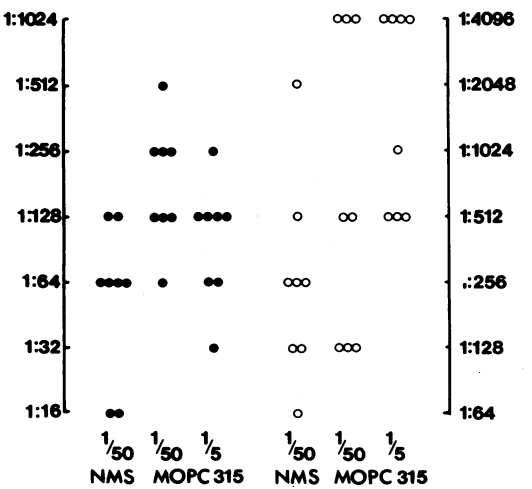

Figure 2. Serum IgG antibody titres to DNP (•) and to ovalbumin (○) in BALB/c mice immunized with 1·3 mg DNP-ovalbumin in saline, after pre-treatment with 0·2 ml of either MOPC-315 serum (1/50 or 1/5) or normal mouse serum (NMS1/50).

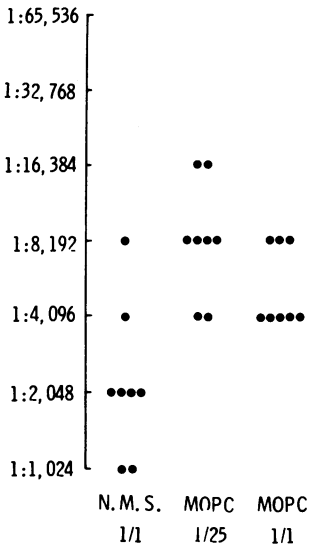

Figure 3. Serum antibody titres to DNP-ovalbumin in BALB/c mice immunized with 1·3 mg DNP-ovalbumin in FCA, after pre-treatment with 0·2 ml of either MOPC-315 serum (1/25 or 1/1) or normal mouse serum (NMS 1/1).

($P=0·01$), and to ovalbumin in the 1 in 5 group ($P<0·01$) (Fig. 2).

In similar experiments on the adjuvantized response, MOPC 315 serum (1/1 and 1/25) or normal mouse serum was first injected, followed by 1·3 mg DNP_4-ovalbumin 8 h later, and 1·3 mg DNP_4-ovalbumin in Freund's complete adjuvant (FCA) intraperitoneally, 10 days later; the animals were bled 2 weeks later, MOPC-315 treated mice had significantly more haemagglutinating antibody to DNP-ovalbumin than did those receiving normal mouse serum (Fig. 3) ($P<0·01$, 1 in 25 group; $P=0·02$, 1 in 1 group). The effect on antibody of ovalbumin was not significant. The addition of 0·1 M 2-mercaptoethalol led to a median reduction of antibody titre of only one in all groups, suggesting that the majority of antibody was IgG. The myeloma protein had no significant effect on the antibody response to ovalbumin (without the hapten).

DISCUSSION

Passive transfer of an IgA myeloma protein with antibody-like affinity for an antigen can both accelerate its elimination as rapidly as serum or isolated serum IgG antibody from hyperimmune mice (Alpers, Steward & Soothill, 1972), and can enhance the subsequent antibody response to DNP and ovalbumin. The elimination of injected MOPC 315 IgA myeloma protein itself is biphasic with half-lives of 3 h and 2·2 days (Jackson, Lemaitre-Coelho & Vaerman, 1977), but virtually all the 'immune elimination' is achieved by between 1 and 4 h clearly faster than even the rapid phase of MOPC 315 decay. We do not know why elimination was faster in the low dose groups. The difficulty of interpretation is increased by the unusual shape of the early phase of the decay curve of ovalbumin in mice, obtained whether DNP labelled or not, and with or without MOPC 315 myeloma protein. Since this study was done Rifai, Small & Ayoub (1978) have reported in abstract that DNP BSA is eliminated by MOPC-315 protein in mice.

It is assumed that IgG antibodies eliminate antigen by complement-dependent opsonization, and phagocytosis by liver and spleen macrophages. Though MOPC 315 binds DNP in an antibody-like way (Eisen *et al.*, 1968) and in certain circumstances IgA may activate both the alternative (Robertson, Caldwell, Castle & Waldmann, 1976; Williams, Slaney, Price & Challacombe, 1976) and the classical pathways (Llida, Fujita, Inal, Sasaki, Kato & Kobayashi, 1976; Burritt, Calvanico, Mehta & Tomasi, 1977), this complement activation is inefficient, therefore it is unlikely to explain this rapid elimination. No receptor has been reported on macrophages for the Fc of IgA, but the cytophilic properties of IgA receptors on lymphocytes (Strober, Hague, Lum, Mann & Henkart, 1978; Lum, Muchmore, Kere, Decker, Koski, Strober & Blaese, 1979) suggest possible mechanisms. We have not studied the site or the mechanisms of the elimination further.

Since some of the MOPC 315 IgA is polymerized, another possible pathway of antigen clearance is the mechanism for transfer of polymerized IgA from blood to bile (Orlans, Peppard, Reynolds & Hall, 1978). This system, which may involve secretory piece attachment, has not been shown to carry antigen too, but it seems a possibility.

Our observation that the antibody response to both DNP and to ovalbumin is increased when animals are immunized with DNP-ovalbumin following injection of MOPC-315 protein, and the similar adjuvantizing effect on B-cell memory response to DNP published since this study was done (Klaus, 1979), present similar problems of mechanism, which may include the systems proposed for such effects of IgG and IgM

antibody (Playfair, 1974; Uhr & Moller, 1968). Hoerlein (1957) and Segre & Myers (1964) have reported an enhancing effect of ingested colostrum, which contains much IgA, on the antibody response to injected antigen in the piglet (Curtis & Bourne, 1971); our studies provide possible mechanism for this.

Ingestion of antigens leads to both a secretory and a systemic antibody response (IgA and other classes), immune exclusion (Walker, Isselbacher & Bloch, 1972) and partial tolerance (Chase, 1946), which occur simultaneously (Swarbrick, Stokes & Soothill, 1979): they are independently genetically controlled (Swarbrick & Stokes, 1979). Passive transfer of orally induced tolerance by serum was prevented by anti IgA antibody (Andre *et al.*, 1975). This raised the attractive unifying hypothesis that IgA complexes with ingested antigens might circulate for a long time, and mediate the tolerance by a suppressive effect. Our demonstration of both rapid antigen elimination and adjuvantizing effect of IgA make this unlikely.

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