Effect of Specific IgM Antibody on Number of IgG Producing Cells in Primary Immune Response (36935)

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(Introduced by E. A. McCulloch)

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The effect of passively administered antibody on the magnitude of the immune response has been studied extensively (1). Specific IgG antibody suppresses the production of both IgM (19S) and IgG (7S) antibody (2). The effect of passively administered IgM antibody is not as clear. It has been reported that specific IgM antibody can suppress, enhance, or have no effect on the immune response, depending on the conditions of the experiment (3–9). The important factors appear to be purity of the IgM preparation, dose and type of antigen, and antigen:antibody ratio. Henry and Jerne (8), using highly purified IgM preparations and suboptimal antigen doses, showed clearly that anti-SRBC IgM antibody can enhance the IgM-plaque forming cell (IgM-pfc) response to SRBC. The results of Dennert (9) suggest that this effect is the result of increased antigen concentration in the spleen. At suboptimal antigen doses the passive administration of IgM antibody would presumably result in specific enhancement of the IgG response as well as the IgM response. To clarify the nature of the enhancement, we have studied the effect of passively administered anti-SRBC IgM antibody on the IgG-pfc response to SRBC.

Preliminary studies showed that for suboptimal antigen doses, the IgG response is small compared to the IgM response. This low response created a problem since the conventional assays for IgG-pfc (10, 11) are not capable of accurately detecting small numbers of IgG-pfc in the presence of larger numbers of IgM-pfc. The problem was overcome by using an adaptation of the IgG-pfc assay developed by Nordin, Cosenza and Hopkins (12), which utilizes the ability of concanavalin A to inhibit IgM plaque formation without affecting IgG plaque formation.

The results reported in this communication demonstrate that there is an increase in the production of IgG-pfc as a result of the passive administration of specific IgM antibody at the time of immunization.

Materials and Methods. Mice. C3H/HeJ mice (purchased from the Jackson Laboratory) and F1 hybrids between C3H/HeOci and C57BL/6JOci (bred at the Ontario Cancer Inst.) were used at approximately 2 mo of age.

Antigens. Sheep red blood cells (SRBC) and horse red blood cells (HRBC), washed three times in phosphate-buffered saline (PBS), and diluted to the appropriate concentration in PBS prior to intravenous injection, were used as antigens.

Assays. IgM-plaque forming cells. Splenic IgM-pfc were assayed using the technique of Jerne, Nordin, and Henry (13), as modified by Kennedy *et al.* (14).

IgG-plaque forming cells. An adaptation of the IgG-pfc assay developed by Nordin, Cosenza and Hopkins (12) was used. Plating of cells was carried out as for the IgM-pfc assay. Following a 1 hr incubation at 37°, 2 ml of concanavalin A (Con A) (Calbiochem) was added to each plate at a concentration of 0.5 mg/ml in PBS. After incubation for 45 min at 37° to inhibit IgM-pfc formation, the Con A was removed and the plates were washed twice with PBS. IgG-pfc were developed by a further 45 min incubation with 2 ml of a mixture of 10% guinea pig serum (as a source of complement) and 2% rabbit anti-mouse IgG serum (the optimum concen-

TABLE I. Sensitivity of IgG-pfc Assay.

Mixture	Relative concn of suspension[a]: IgM-pfc	IgG-pfc	Detected IgM-pfc	IgG-pfc
A	1[b]	1[b]	98	99
B	2	1	162	86
C	4	1	461	100
D	8	1	775	98
E	16	1	1532	90

[a] The IgM-pfc suspension contained no detectable IgG-pfc. The IgG-pfc suspension contained about 7% IgM-pfc; see text for details.

[b] Corresponds to 0.0005 of a spleen.

tration of this serum for development of IgG-pfc as determined in other experiments). Preliminary experiments on the effect of Con A showed that at the concentration used (0.5 mg/ml) it inhibited more than 95% of IgM-pfc and had no detectable inhibitory effect on IgG-pfc. Thus, under these conditions, the assay detects primarily IgG-pfc. The sensitivity of the assay was tested as follows. Two spleen cell suspensions, one containing only IgM-pfc (from early primary response) and one containing greater than 90% IgG-pfc (secondary response) were mixed together in various proportions in such a way that the number of expected IgG-pfc was kept constant while the number of IgM-pfc was varied. The mixtures were then assayed for both IgM-pfc and IgG-pfc as described above. The results of this test (Table I) demonstrate that the assay can detect accurately the number of IgG-pfc, even in a 16-fold excess of IgM-pfc; these data also confirm the observation of Nordin, Cosenza and Hopkins (12) that Con A specifically inhibits IgM-pfc and not IgG-pfc.

Preparation of IgM. IgM antibody was isolated from the serum of C3H mice collected 5 days after primary immunization with 4×10^8 SRBC. The serum was processed in one of three ways:

A. Euglobin precipitation. This procedure results in the preferential precipitation of IgM antibodies. The method used was that described by Henry and Jerne (8). The serum was diluted tenfold in distilled water and the pH was adjusted to 5.5. A precipitate formed which was collected and washed in the same volume of distilled water at pH 5.5. The precipitate was then dissolved in PBS to the original serum volume. This procedure was repeated thre times.

B. Sephadex chromatography. The serum was separated on a Sephadex G-200 column equilibrated with Tris–HCl at pH 7.5, ionic strength 0.15. The IgM peak was pooled, concentrated by pressure dialysis, and passed through the column a second time. The IgM peak was pooled and dialyzed against PBS.

C. Euglobin precipitation and Sephadex chromatography. The IgM fraction was first isolated by a single euglobin precipitation. The precipitate was dissolved in PBS and passed through a G-200 column. A single protein peak was obtained in the region where IgM elutes. The peak was pooled and dialyzed against PBS.

Results and Discussion. Preliminary experiments verified the published reports that passive administration of anti-SRBC IgM antibody enhances the IgM-pfc response to a suboptimal dose of SRBC. We then extended these studies to look at the effect of anti-SRBC IgM antibody on the primary IgG-pfc response to SRBC.

Groups of mice received by intravenous injection various concentrations of each of the three anti-SRBC IgM preparations, followed 15–60 min later by a suboptimal antigen dose of 10^6 SRBC. At the peak of the IgG-pfc response to this antigen dose. (Day 13), the mice were sacrificed and their spleens were assayed for IgG-pfc as described in Materials and Methods. The results of these experiments are shown in Fig. 1. The IgG-pfc response increased from a control value (no IgM injected) of about 200 pfc up

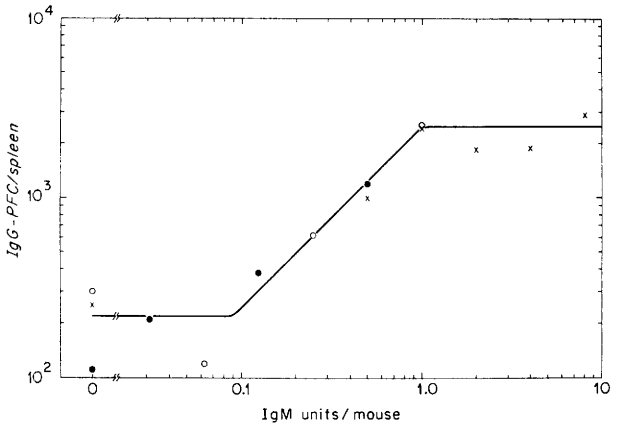

FIG. 1. The effect of passively administered IgM antibody on the IgG-pfc response. Groups of mice (10 per group) received intravenous injections of various concentrations of the IgM preparations, diluted in PBS, in a volume of 0.2 or 0.5 ml. Control groups received equal volumes of PBS. Fifteen to 60 min later the mice received an intravenous injection of 10^6 SRBC in 0.5 ml of PBS. Spleens were assayed for IgG-pfc on Day 13 (optimum time of assay for IgG-pfc at this antigen dose). Data obtained with IgM prepared by the three methods outlined in Materials and Methods are shown: (○) Preparation A (recipients are C3H); (X) Preparation B (recipients are C3H); (●) Preparation C (recipients are F1 hybrids of C3H × C57BL). Since the three IgM samples were prepared differently, it is difficult to compare them with respect to standard parameters. Therefore, the lowest concentration of IgM which increased the IgG-pfc response to 2500 pfc was assigned the value of 1 IgM unit for purposes of comparison (1 IgM unit/ml is roughly equivalent to a hemolytic titer of 2^7).

to a maximum of 2500 pfc. Below the plateau level, the degree of enhancement was proportional to the concentration of IgM administered.

Experiments were carried out to investigate the specificity of the enhancement of the IgG response. IgM antibody against horse erythrocytes (HRBC) was prepared in the

TABLE II. Characterization of IgM Enhancement of the IgG Response.

	Group	IgM[a]	Antigen[b]	IgG-pfc/spleen[c]
A. Comparison of effect using two different antigens	1	—	10^6 SRBC	110 (80–150)
	2	anti-SRBC	10^6 SRBC	1200 (970–1500)
	3	—	10^6 HRBC	140 (90–210)
	4	anti-HRBC	10^6 HRBC	650 (520–820)
B. Specificity of effect	1	—	10^6 SRBC	40 (30–60)
	2	anti-SRBC	10^6 SRBC	1200 (1060–1350)
	3	anti-HRBC	10^6 SRBC	120 (90–170)
C. Effect at high antigen dose	1	—	10^8 SRBC	5600 (4900–6400)
	2	anti-SRBC	10^8 SRBC	4700 (3900–5600)

[a] Mice in experimental groups received 0.28 mg protein in 0.5 ml PBS intravenously. Control animals received 0.5 ml of PBS. The anti-SRBC IgM used was preparation C.

[b] All immunizations were given intravenously in 0.5 ml of PBS 1 hr after injection of IgM (or PBS).

[c] Mice were assayed for IgG-pfc against the immunizing antigen on Day 13 when an antigen dose of 10^6 erythrocytes was used, and on Day 10 when a dose of 10^8 erythrocytes was used. These times were found to be optimum for the respective antigen doses. The values shown are the geometric means; 1 SE on each side of the mean is given in parentheses.

same manner as preparation C against SRBC. Equal protein concentrations [determined by the Lowry *et al.* method (15)] of each preparation were administered to groups of mice (controls received PBS) followed 1 hr later by the respective antigen (10^6 erythrocytes). The results, tabulated in Table II-A, show that enhancement was obtained for HRBC as well as for SRBC. In order to test specificity of the effect, mice were given equal concentrations of anti-SRBC IgM or anti-HRBC IgM (controls received PBS). The mice were then immunized with SRBC and assayed for IgG-pfc against SRBC. The results, shown in Table II-B, demonstrate that anti-SRBC IgM enhanced the response while anti-HRBC IgM had little effect. Thus, the IgM enhancement of IgG-pfc appears to be antigen specific.

Henry and Jerne (8) found that IgM enhancement of the IgM-pfc response occurred only at suboptimal antigen doses. We, therefore, tested the effect of anti-SRBC IgM on the response to 10^8 SRBC. The results are presented in Table II-C and show that, at this dose of SRBC, there is no enhancement.

We have shown that IgM antibody enhances the immune response of mice to erythrocyte antigens as measured by the production of IgG antibody forming cells. The effect is specific with regard to antigen and occurs only when suboptimal antigen doses are used. This observation completes the list of all possible combinations: IgM antibody can specifically enhance both IgM and IgG responses whereas IgG specifically suppresses both responses. Our results are compatible with the theory of Dennert (9) that IgM antibody acts by increasing the concentration of antigen in the spleen. Further evidence for this model comes from our failure (unpublished data), and that of Dennert (9), to obtain IgM-mediated enhancement *in vitro*. The site of action of IgM enhancement, therefore, appears to differ from that of IgG inhibition, since the latter occurs *in vitro* (16, 17) and may be the result of a direct effect on one of the cells involved in the initiation of an immune response (18).

Summary. Passive administration of antibody during the course of an immune response can be used as a model system for studying physiological mechanisms of feedback control by circulating antibody. We have studied the effect of passively administered specific IgM antibody on the primary IgG response of mice to heterologous erythrocytes. These studies show that IgM antibody can enhance the IgG response to a suboptimal dose of the erythrocyte antigen. The effect is specific with regard to antigen and does not occur at an optimal antigen dose.

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