Effects of IgM on the *in Vivo* and *in Vitro* Immune Response (37435)

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(Introduced by J. Salk)

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An immune response to heterologous erythrocytes requires the cooperation of bone marrow derived lymphocytes (B cells),[2] with thymus derived lymphocytes (T cells) (1, 2). Recent experiments suggest that these cooperating cells interact with each other by means of a variety of humoral factors (3, 4). In addition, antibody can regulate an *in vivo* immune response by a feedback mechanism (5). Both inhibition and stimulation have been described: IgM antibody for example usually stimulates whereas IgG antibody usually inhibits, but can stimulate if given in low doses (6). Because there are reports showing that carrier specific antibody may mimic T cell priming (7), and there are indications that monomeric IgM is either secreted by T cells (8) or can be found on T cell membranes (9), experiments to find out whether purified IgM can substitute for the T cell function are of interest. It is shown in this report that it cannot. In particular, while specific anti-SRBC IgM is stimulatory in intact mice, it fails to stimulate under T cell depleted conditions, *in vitro* or *in vivo*. Thus, it is concluded that T cells are required for this stimulation and that IgM cannot replace them.

Materials and Methods. Outbred NMRI mice were obtained from Ivanovas Kissleg, Germany, and $B_6D_2F_1$ mice as well as CBA mice were obtained from Jackson Laboratories, Bar Harbor, ME.

Animals were thymectomized at the age of 3–4 wk, irradiated with 1000 R using a Siemens X-ray tube, and protected with 10^7 syngeneic fetal liver cells. Serum titrations and plaque assays were performed as described previously (6). Anti-μ IgG was prepared by injecting rabbits with 1 mg IgM (kindly provided by B. Askonas) in Freund's complete adjuvant followed in 4 wk by an intravenous boost with 500 μg IgM. The IgG fraction was purified by DEAE chromatography and the anti-μ immunoadsorbent was prepared following the method of Avrameas and Ternynck (11). The SRBC immunoadsorbent (10) and the anti-SRBC IgM (6) were prepared as described previously. IgM was adsorbed to the SRBC or anti-μ immunoadsorbent. After washing in BSS the IgM was desorbed from the immunoadsorbent by adding 1 *M* Tris. After centrifugation the supernatant was pipetted off and dialyzed against BSS. The *in vitro* immune response to SRBC was assayed as described by Mishell and Dutton (12).

Results and Discussion. Anti-SRBC IgM antibody enriched by successive steps of acid precipitation, G200-Sephadex filtration and utracentrifugation (6) still contains components other than IgM as can be shown by sucrose gradient centrifugation and column electrophoresis (unpublished data). Therefore to further confirm specific anti-SRBC IgM as the stimulatory substance a preparation was purified by either anti-μ or -SRBC immunoadsorbents. Table I shows that the material desorbed from either immunoadsorbent efficiently enhances the immune response to SRBC in intact mice. This finding, together with the previously demonstrated specificity of action of anti-SRBC IgM (6,

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[2] Abbreviations used in this paper: B cells = bone marrow-derived lymphocytes; T cells = thymus-derived lymphocytes; SRBC = sheep red blood cells; BSS = balanced salt solution; PBS = phosphate buffered saline; PFC = plaque forming cells.

TABLE I. Effect of IgM Purified by SRBC or Anti-μ Immunoadsorbent on the *in Vivo* Immune Response.[a]

IgM injected	Titer of injected IgM/agglutinating hemolytic		PFC/10^6
No IgM	—	—	28
SRBC desorbed IgM	2^5	2^6	337
Anti-μ desorbed IgM	2^4	2^5	248

[a] Portions (0.1 ml) of SRBC and anti-μ IgG desorbed IgM were injected with 4×10^5 SRBC into groups of 5 NMRI mice. The plaque assay was done on Day 6. The arithmetic mean of PFC per 10^6 spleen cells of groups of individually assayed spleens is given.

13) implicates the SRBC specific 19S IgM as the stimulatory substance.

The stimulatory activity of IgM depends on IgM dose injected (5, 6), time interval between IgM and antigen injection, as well as antigen dose. In Table II, Expts 1 and 2 show that IgM can be injected at least 3 days prior to the antigen and still be effective, though the stimulatory activity slowly disappears. IgM however does not stimulate if injected 2 hr after the antigen. This probably indicates that it is the antigen–antibody complexes found in the blood that are responsible for the stimulatory effect of IgM. A clue to this is the fact that 98% of the ^{51}Cr labeled SRBC injected into normal animals disappeared from the bloodstream within 10 min (13). If IgM stimulates by antigen concentration in the spleen, the stimulation might only be evident at suboptimal antigen concentrations. In support of this, Table III shows that a significant stimulation of hemagglutinin titer is only found if low doses of SRBC (5×10^5–5×10^7 SRBC/mouse) are injected. However, no stimulation is found if as much as 5×10^8 SRBC are given. If IgM stimulates by concentrating antigen in the spleen, the IgM stimulated response should occur earlier than the unstimulated response. Therefore, the

TABLE II. Ability of Circulating IgM to Stimulate the Immune Response and Time Course of the IgM Stimulation.[a]

Expt	Material injected 1st	Material injected 2nd	Time interval between the injections (hr)	PFC/10^6	Day of assay
1	—	4×10^5 SRBC	—	52	6
	0.1 ml IgM	4×10^5 SRBC	0	457	6
	0.1 ml IgM	4×10^5 SRBC	24	324	6
	0.1 ml IgM	4×10^5 SRBC	72	152	6
2	—	4×10^5 SRBC	—	11	6
	4×10^5 SRBC	0.1 ml IgM	0	115	6
	0.1 ml IgM	4×10^5 SRBC	2	103	6
	4×10^5 SRBC	0.1 ml IgM	2	5	6
3	—	4×10^5 SRBC	—	2	3
	—	4×10^5 SRBC	—	2	4
	—	4×10^5 SRBC	—	12	5
	—	4×10^5 SRBC	—	27	6
	—	4×10^5 SRBC	—	12	7
	0.2 ml IgM	4×10^5 SRBC	0	13	3
	0.2 ml IgM	4×10^5 SRBC	0	31	4
	0.2 ml IgM	4×10^5 SRBC	0	425	5
	0.2 ml IgM	4×10^5 SRBC	0	530	6
	0.2 ml IgM	4×10^5 SRBC	0	111	7

[a] Expt 1. Groups of 5 NMRI mice were injected with IgM purified as described (6); the hemolytic titer was 2^8 and the agglutination titer was 2^6. Expts 2 and 3: experimental conditions as in Expt 1.

TABLE III. Effect of Increasing Antigen Dose on the IgM Stimulation.[a]

Dose injected (SRBC)	Log 2 of the serum titers on Day 6	
	Without IgM	With IgM
5×10^5	4.5	6.8
5×10^6	5.5	7.9
5×10^7	7.3	8.5
5×10^8	8.3	8.6

[a] Groups of 4 NMRI mice were injected with 0.1 ml of IgM (same preparation as Table II) and increasing amounts of SRBC. The means of the log 2 hemolytic titers of the IgM injected and control animals are given.

IgM stimulated and unstimulated response to 4×10^5 SRBC was assayed daily between Days 3 and 7 after injection. As shown in Table II, Expt 3, the IgM enhanced response rises steeply from Days 3 to 5, whereas in the response without injected IgM the rise in response is delayed until Day 4. IgM injection therefore results in an earlier initiation of the response, without much change in the time of maximal response.

To determine whether 19S anti-SRBC IgM could substitute for the helper function of T cells, its action was tested in thymectomized mice. When low doses of SRBC (4×10^5 cells/mouse) were injected with IgM into groups of such mice and the spleens were assayed for PFC on Day 5, no detectable stimulation by IgM was observed. In other experiments, higher doses of SRBC were used. At a dose of 4×10^7 SRBC/mouse, which in the normal mouse can be enhanced with IgM (Table III), no such enhancement can be seen in thymus depleted mice (Table IV). This shows that IgM cannot substitute for the T cell helper function in thymectomized mice, at least for the doses of SRBC and IgM tested in these experiments.

A more complete titration of different IgM doses was performed *in vitro*. Aliquots of IgM in dilutions between 1:2 to 1:2048 were mixed with normal spleen cells and SRBC in Mishell–Dutton type cultures (12), and the response was assayed for 5 days later. As shown in Expt 1, Table V, IgM aliquots with agglutinin titers between 2^2 and 2^6 (dilutions 1:2 to 1:32) severely inhibit the immune response. This is different from the *in vivo* situation where the same IgM doses exert considerable stimulation (Tables I–IV). The effective dose of IgM given *in vivo* and *in vitro* is considered to differ not more than by a factor of two, since a mouse has a blood volume between 1 and 2 ml (14). It is noteworthy however that at very low IgM doses, a very small but reproducible stimulation of the *in vitro* immune response is consistently observed. To titrate IgM under T cell depleted conditions, spleen cells were treated with anti-Θ serum and complement and used for *in vitro* experiments. Such cell preparations failed to respond to SRBC unless sensitized T cells were added (Expt 2, Table V). It is shown that low doses of IgM, which exert a slight stimulation in normal spleen cells, also stimulate the anti-Θ treated

TABLE IV. Effect of IgM in Thymectomized Mice.[a]

Animals	Injection of			PFC/10^6
	SRBC dose	IgM	Thymus cells	
CBA thymectomized	4×10^7	—	—	14
	4×10^7	0.1 ml	—	15
	4×10^7	—	2×10^8	615
	4×10^7	0.1 ml	2×10^8	519
CBA normal	4×10^6	—	—	200
	4×10^6	0.1 ml	—	920

[a] Groups of 5 normal and 5 thymectomized CBA mice were injected with SRBC and then with IgM either alone or together with syngeneic thymus cells as indicated. The number of PFC was assayed on Day 5. The IgM used had a hemolytic titer of 2^6 and an agglutinating titer of 2^4 (same preparation as Table II).

TABLE V. Effect of IgM on the *in Vitro* Immune Response of Normal and Anti-θ Treated Spleen Cells.[a]

	Dilution of IgM or T cells added	SRBC antigen	PFC/10^6
Expt 1	No IgM	—	77
Normal spleens	No IgM	+	3030
	1:2	—	1
	1:2	+	1
	1:8	—	77
	1:8	+	76
	1:32	—	119
	1:32	+	234
	1:128	—	101
	1:128	+	2014
	1:512	—	222
	1:512	+	5939
	1:2048	—	134
	1:2048	+	4260
Expt 2	No IgM	—	4
Anti-θ treated spleens	No IgM	+	2
	3×10^6 T cells	—	128
	3×10^6 T cells	+	1080
	1:4	+	2
	1:16	+	3
	1:64	+	3
	1:256	+	2
	1:1024	+	31
	1:4096	+	83

[a] Expt 1. B6D2F1 spleen cells were cultured with SRBC and 0.1 ml IgM of the dilutions given. Expt 2. B6D2F1 spleens were treated with anti-θ and guinea pig complement (15) and cultured with IgM or SRBC sensitized T cells (15). The IgM preparation purified as in Table II had a hemagglutination titer of 2^7. IgM concentrations lower than those shown did not exert any significant stimulation.

spleen cells. However, the response brought about by IgM is more than 10-fold lower than the response obtained when sensitized T cells are added to the anti-Θ treated spleen cell cultures. This finding implies that SRBC specific 19S IgM cannot mimic the T cell function, perhaps because complete stimulation requires the participation of additional T cell factors. Monomeric IgM prepared from 19S IgM (J. D. Watson, unpublished data) does not stimulate B spleens *in vitro* better than 19S IgM. Specific 19S IgM isolated from mouse serum, therefore, is not able to substitute for the T cell function in either *in vivo* or *in vitro*.

Summary. The effect of sheep erythrocyte specific IgM on the *in vitro* and *in vivo* immune response to SRBC is described. Kinetic data resulting from separate injection of antibody and antigen suggest that the stimulatory effect is mediated by an antigen–antibody complex formed in the peripheral circulation, and subsequently trapped in the spleen. If an optimal antigen dose is chosen, then IgM given with it does not improve the response, thus supporting the view that IgM works by increasing the spleen concentration of antigen given at suboptimal doses. *In vitro* studies with normal spleen cells show that high concentrations of IgM inhibit the sheep erythrocyte response. In experiments performed *in vivo* with the thymectomized animals, IgM does not substitute for the T cell component. However, *in vitro* experiments with T cell deficient spleen cells do show a small improvement of the SRBC response

when low doses of IgM are given. This IgM stimulated response is much lower than the response obtained when B spleen cells are mixed with sensitized T cells.

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