Brief Definitive Report

COMPLEMENT ACTIVATION IS REQUIRED FOR IgM-MEDIATED ENHANCEMENT OF THE ANTIBODY RESPONSE

By BIRGITTA HEYMAN,* LARS PILSTRÖM,‡ AND MARC J. SHULMAN§

*From the *Department of Immunology, Uppsala University, S-751 23 Uppsala, Sweden; the ‡Department of Zoophysiology, Uppsala University, Uppsala, Sweden; and the §Department of Immunology, University of Toronto, Toronto, Ontario, Canada*

The ability of IgM antibodies to specifically enhance the thymus-dependent humoral immune response to particulate antigens is well documented (1–5). The requirements for the potentiation by IgM are that a suboptimal dose of the antigen is used (1) and that an in vivo system is used (5). The IgM-mediated enhancement requires presence of T cells (6).

In the present study the importance of activation of complement factors for the ability of IgM to induce enhancement was investigated. We found that mutant IgM antibodies unable to activate complement lack the enhancing properties of the wild-type IgM. Moreover, IgM cannot enhance the immune response in mice depleted of complement by treatment with cobra venom factor. The significance of these findings with regard to the mechanism behind IgM-mediated enhancement and cobra venom factor (CVF)-induced immunosuppression is discussed.

Materials and Methods

Mice. Male CBA/Ca and AKR mice, 10–16 wk old, from A-lab, Stockholm, Sweden and our own breeding facilities were used.

Antigens. SRBC and HRBC were purchased from the National Veterinary Institute, Håtunaholm, Sweden. Erythrocytes were stored in sterile Alsevers solution at 4°C. TNP was coupled to SRBC according to Rittenberg and Pratt (7). The amount of TNP coupled is given as microgram/milliliter packed erythrocytes.

Antibodies. Polyclonal IgM was derived from sera of CBA/Ca or AKR mice 6 d after an intraperitoneal injection of 10% SRBC in PBS and prepared as described (3). The hybridomas producing the wild-type and mutant 13 IgM-anti-TNP antibodies are described elsewhere (8). mAbs used in this study were isolated from cell culture supernatants by ammonium sulphate precipitation followed by gel filtration on a Sephacryl S-300 column.

Hemagglutination/Hemolysis. The hemagglutination and hemolytic titers are defined as the highest antibody dilution able to agglutinate or lyse a 0.25% suspension of SRBC or SRBC-TNP_{100} in the presence of guinea pig complement (lysis only) after a 1-h incubation at 37°C.

CVF Treatment. Mice were injected intraperitoneally with four doses of 0.1 ml of 100 U/ml CVF from *Naja haje* (Cordis Laboratories Inc., Miami, Florida) during 24 h. Control groups received PBS in the same regimen. All mice were bled individually from the tail 7 d before

This work was funded by the Swedish Medical Research Council, the Swedish Society for Medicine, and by grants from the Swedish Board for Technical Development. Address correspondence to B. Heyman, Department of Immunology, Uppsala University, Box 582, BMC, S-751 23 Uppsala, Sweden.

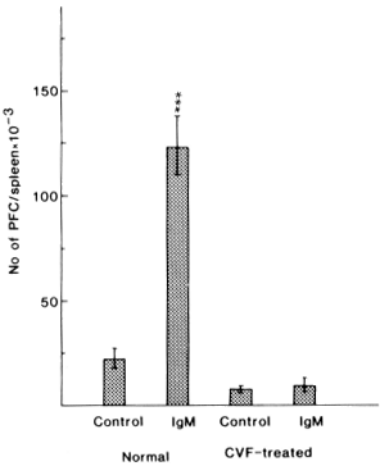

FIGURE 1. Effect of complement depletion on IgM-induced enhancement of the antibody response in mice. Two groups of CBA/Ca mice were injected intraperitoneally with CVF. Two control groups received PBS in the same regimen. 2 h after the last CVF injection all mice were bled from their tails in order to titer C3. Another 2 h later, one CVF group and one control group received intravenous injections of 0.1 ml murine, polyclonal IgM-anti-SRBC (hemolytic titer 2,048). 1 h later all groups were immunized intravenously with 4×10^5 SRBC (10 mice in two different experiments) together with 2×10^5 HRBC (horse erythrocytes). (***) $p < 0.001$ vs. the respective control group. Mice injected only with IgM-anti-SRBC generated 65 PFC/spleen (not shown). No significant enhancement of PFC anti-HRBC was found (not shown).

Sera from the individual mice (bled 7 d before and 2 h after CVF treatment) were assayed for C3 by radial immunodiffusion against goat anti-mouse C3 antibodies. Mean titer was 6.3 ± 1.3 (SEM) after and 45.6 ± 6.5 before CVF treatment.

the first injection and 2 h after the last. C3 levels in sera were assayed by radial immunodiffusion using a goat anti-mouse C3 antiserum (CooperBiomedical, Inc., Malvern, PA).

Immunizations. Groups of at least four mice were immunized as described in the figure legends.

Enzyme-linked Immunosorbent Assay. SRBC-specific IgG antibodies in sera were assayed in an ELISA, as described elsewhere (9).

PFC Assay. A modified version of the Jerne hemolytic plaque assay was performed (3). The p values were estimated by Student's t test.

Results and Discussion

In vivo administration of CVF causes transient depletion of plasma C3 to <5% of normal due to activation of C3 via the alternative pathway (10). Mice were treated with CVF and then challenged with polyclonal IgM-anti-SRBC and SRBC. This procedure abrogated the five- to sixfold enhancing effect on the direct PFC response of IgM seen in normal mice immunized with 4×10^5 SRBC (Fig. 1). Moreover, no elevation of the IgG-anti-SRBC levels in sera of CVF- and IgM-treated mice was detected (Table I). CVF from *Naja haje* does not deplete complement factors that act subsequent to C3 in the complement pathway (11). These data therefore

TABLE I

IgG-Anti-SRBC Response in Mice Treated with CVF before Specific IgM-induced Enhancement

Treatment	Dose of SRBC	A_{405} (SD)	Number of mice	p vs. control
–	4×10^5	0.177 (0.071)	10	
IgM	4×10^5	0.429 (0.157)	10	0.001
CVF	4×10^5	0.137 (0.035)	10	
CVF + IgM	4×10^5	0.165 (0.064)	10	NS

Sera from the same mice as described in Fig. 1 were prepared on the day of PFC test and stored at – 20°C. They were tested for IgG-anti-SRBC in 1:10 dilutions in an ELISA. Values are expressed as absorbance at 405 nm.

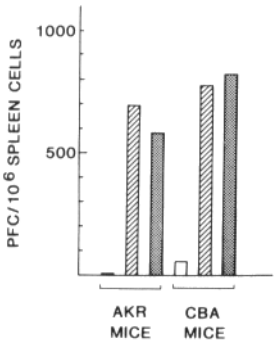

FIGURE 2. Efficient IgM-mediated enhancement in C5⁻ AKR mice. CBA/Ca or AKR mice were injected intravenously with IgM-anti-SRBC (hemolytic titer 4,096) either from CBA/Ca (*stippled bars*) or AKR (*striped bars*) sera followed after 1 h by 4×10^5 SRBC. Control groups (*open bars*) received antigen alone. The PFC numbers in IgM-injected groups were always significantly higher than controls ($p < 0.001$).

indicate that the early complement factors are crucial in the enhancing process. To confirm this, AKR mice, which are genetically deficient of C5 (12), were injected with SRBC-specific IgM and SRBC and the enhancement of the anti-SRBC response in these mice was compared with that in similarly treated CBA/Ca mice having normal C5 levels. It is clearly demonstrated that IgM is a potent enhancer also of the humoral immune response in AKR mice (Fig. 2).

Next, we compared the effects on the anti-SRBC response of a monoclonal TNP-specific IgM and a mutant of this antibody, IgM13, which has <1% the normal capacity to activate complement due to a single amino acid substitution (8). Mice were immunized with SRBC-TNP with or without IgM-anti-TNP of either the wild or mutant type, with the same hemagglutinating titers. Since IgM is known to enhance the response against all different epitopes on an antigen equally well (3), the PFC response against SRBC was measured. The wild-type antibody enhanced the response in a dose-dependent fashion, whereas the mutant antibody failed to induce any significant enhancement (Fig. 3).

In a model for IgM-mediated enhancement of the humoral immune response there

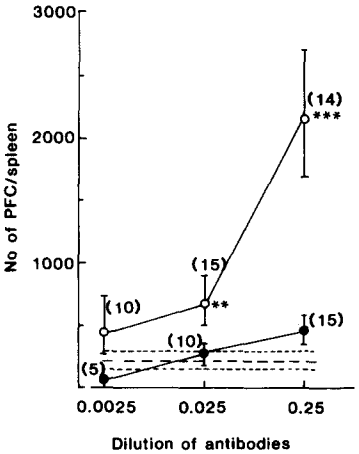

FIGURE 3. Effect of complement activating and nonactivating IgM-anti-TNP antibodies on the antibody response in mice. Groups of CBA/Ca mice (as in Fig. 1) were injected intravenously with 0.1 ml of either PBS (*dotted line*), wild-type IgM (○) or mutant 13 IgM (●). 1 h later all groups were immunized intravenously with 4×10^4 SRBC-TNP_5 in 0.1 ml PBS. The undiluted IgM preparations both had hemagglutinating titers of 1:6,400 against SRBC-TNP_{100}. The wild-type antibodies had a hemolytic titer of 1:20,480 against SRBC-TNP_{100}. No hemolytic activity could be detected in the mutant preparation. Direct PFC-anti-SRBC were assayed 6 d after immunization. The results (pooled from three experiments) are expressed as the geometrical mean of each group and the bars denote antilog of SEM. Numbers in parentheses indicate the number of animals. (***) $p < 0.001$ and (**) $p < 0.01$ in comparison to the control group.

are two main concepts to consider. The first is the central role of complement in induction of immunological memory and normal humoral responses (reviewed in references 13, 14). These effects have been ascribed to C3 (14). Also, patients with C4 deficiency respond poorly to T-dependent antigens and do not develop immunological memory (15). Our results on the effect of CVF treatment on the IgM enhancement are most likely due to decreased C3 levels since we used CVF from Egyptian cobra (*Naja haje*) and IgM is an efficient enhancer of the anti-SRBC response in AKR mice. The second concept is the important role of the spleen in immune responses affected by C3 and/or IgM potentiation. Complement is essential for efficient localization of antigen to the spleen (16, 17). The IgM-mediated enhancement is paralleled by an increased level of SRBC in the spleen (2). Splenectomized mice (Heyman, B., unpublished data) as well as patients with hereditary aplasia of the spleen (18) are poor responders to intravenously administered SRBC, the route normally used in IgM enhancement studies.

We propose the following model for IgM enhancement of responses against particulate antigens. In the spleen, complexes of antigen–IgM–activated C3 fragments are trapped more efficiently than antigen alone on the follicular dentritic cells (FDC), which carry C3b receptors (19). The trapped immune complexes then capture B cells carrying antigen-specific as well as C3 receptors. Crosslinked C3 is known to stimulate activated B cells to proliferation and Ig secretion (20). Additional help to these B cells may be provided by antigen-specific T helper cells that are activated more efficiently due to increased concentrations of antigen, and possibly by IL-1 secretion by the FDC.

Thus, the mechanism behind IgM-mediated enhancement would be a more efficient presentation of antigen and more efficient activation of antigen sensitive B cells. This explains why the same fine specificity (3) and isotype distribution (21) are found in IgM-enhanced and control responses. Moreover, IgM is known to potentiate (1) and CVF to suppress (22) the immune response only to suboptimal antigen doses.

Interestingly, IgG has been shown to enhance the primary response (23) as well as the induction of memory (17) to soluble antigens. This has been ascribed to the ability of the IgG antibodies to activate complement, and a hypothesis similar to ours was proposed to explain the results (17).

Summary

The ability of IgM antibodies to specifically enhance the thymus-dependent humoral immune response to particulate antigens is well documented. We have used two approaches to test whether complement factors play a role in this process. First, mice were depleted of C3 by treatment with cobra venom factor (CVF) and then immunized with SRBC with or without IgM-anti-SRBC. CVF treatment severely impaired the capacity of IgM to induce an enhanced anti-SRBC response. Moreover, it was shown that IgM can potentiate the response in C5-deficient AKR mice, thus demonstrating that the complement factors acting before C5 are the crucial ones. A second test compared the enhancing properties of two monoclonal IgM-anti-TNP antibodies where, because of a point mutation in the μ chain constant region, one of the antibodies is impaired in its capacity to activate complement. We show that the mutant antibody lacks the enhancing properties of the wild-type IgM.

Activation of C3 by IgM antibodies as well as localization of antigen in the spleen

seem to be necessary steps in the IgM-mediated enhancement of antibody responses. Our data offer an explanation to the immunosuppression described in CVF-treated animals as well as the low humoral immune responses in certain hereditary complement deficiencies. It is suggested that IgM indeed has an important physiological function in enhancing antibody responses to foreign substances.

Excellent technical assistance by Ms I. Brogren and critical review of the manuscript by Drs. J. Andersson, M. E. Jakobson, and H. Wigzell are gratefully acknowledged.

Received for publication 17 February 1988 and in revised form 21 March 1988.

References

1. Henry, C., and N. Jerne. 1968. Competition of 19S and 7S antigen receptors in the regulation of the primary immune response. *J. Exp. Med.* 128:133.
2. Dennert, G. 1971. The mechanism of antibody induced stimulation and inhibition of the immune response. *J. Immunol.* 106:951.
3. Heyman, B., G. Andrighetto, and H. Wigzell. 1982. Antigen-dependent IgM-mediated enhancement of the sheep erythrocyte response in mice. Evidence for induction of B cells with specificities other than that of the injected antibodies. *J. Exp. Med.* 155:994.
4. Harte, P. G., A. Cooke, and J. H. L. Playfair. 1983. Specific monoclonal IgM is a potent adjuvant in murine malaria vaccination. *Nature (Lond.).* 302:256.
5. Schrader, J. W. 1973. Regulation of the immune response by IgM antibody: a paradoxical suppression of the in vitro primary immune response to sheep erythrocytes by IgM. *Aust. J. Exp. Biol. Med. Sci.* 51:333.
6. McBride, R. A., and L. W. Schierman. 1973. Thymus dependency of antibody-mediated helper effect. *J. Immunol.* 110:1710.
7. Rittenberg, M. B., and K. L. Pratt. 1969. Anti-trinitrophenyl (TNP) plaque assay. Primary response of BALB/c mice to soluble and particulate immunogens. *Proc. Soc. Exp. Biol. Med.* 132:575.
8. Shulman, M. J., C. Collins, N. Pennell, and N. Hozumi. 1987. Complement activation by IgM: evidence for the importance of the third constant domain of the u heavy chain. *Eur. J. Immunol.* 17:549.
9. Heyman, B., G. Holmquist, P. Borwell, and U. Heyman. 1984. An enzyme-linked immunosorbent assay for measuring anti-sheep erythrocyte antibodies. *J. Immunol. Methods.* 68:193.
10. Pepys, M. B. 1975. Studies in vivo of cobra factor and murine C3. *Immunology.* 28:369.
11. von Zabern, I., B. Hinsch, H. Przyklenk, G. Schmidt, and W. Vogt. 1980. Comparison of Naja n. naja and Naja h. haje cobra venom factors: correlation between binding affinity for the fifth component of complement and mediation of its cleavage. *Immunobiology.* 157:499.
12. Cinader, B., S. Dubiski, and A. C. Wardlaw. 1964. Distribution, inheritance and properties of an antigen, MuB1, and its relation to hemolytic complement. *J. Exp. Med.* 120:897.
13. Pepys, M. B. 1974. Role of complement in induction of antibody production in vivo. Effect of cobra factor and other C3-reactive agents on thymus-dependent and thymus-independent antibody responses. *J. Exp. Med.* 140:126.
14. Böttger, E. C., and D. Bitter-Suermann. 1987. Complement and the regulation of humoral immune responses. *Immunol. Today.* 8:261.
15. Jackson, C., H. D. Ochs, and R. J. Wedgwood. 1979. Immune response of a patient with deficiency of the fourth component of complement and systemic lupus erythematosus. *N. Engl. J. Med.* 300:1124.

16. Papamichail, M., C. Gutierrez, P. Embling, E. J. Holborow, and M. B. Pepys. 1975. Complement dependence of localization of aggregated IgG in germinal centers. *Scand. J. Immunol.* 4:343.
17. Klaus, G. G. B. 1979. Generation of memory cells. III. antibody class requirements for the generation of B-memory cells by antigen-antibody complexes. *Immunology.* 37:345.
18. Kevy, S. V., M. Tefft, G. F. Vawter, and F. S. Rosen. 1968. Hereditary splenic hypoplasia. *Pediatrics.* 42:752.
19. Heinen, E., D. Radoux, C. Kinet-Denoel, M. Moeremans, J. de Mey, and L. J. Simar. 1985. Isolation of follicular dentritic cells from human tonsils and adenoids. III. Analysis of their Fc receptors. *Immunology.* 54:777.
20. Erdei, A., F. Melchers, T. Schulz, and M. Dierich. 1985. The action of human C3 in soluble or cross-linked form with resting and activated murine B lymphocytes. *Eur. J. Immunol.* 15:184.
21. Heyman, B., M. V. Hobbs, and W. O. Weigle. 1985. IgM-mediated enhancement of in vivo anti-sheep erythrocyte antibody responses: isotype analysis of the enhanced responses. *Cell. Immunol.* 92:134
22. Böttger, E. C., T. Hoffmann, S. Metzger, U. Hadding, and D. Bitter-Suermann. 1986. The role and mechanism of cobra venom factor-induced suppression of the humoral immune response in guinea pigs. *J. Immunol.* 137:1280.
23. Coulie, P., and J. Van Snick. 1985. Enhancement of IgG anti-carrier responses by IgG2-anti-hapten antibodies in mice. *Eur. J. Immunol.* 15:793.