Proc. Natl. Acad. Sci. USA
Vol. 77, No. 2, pp. 1125–1128, February 1980
Immunology

IgM antibodies induce the production of antibodies of the same specificity

(immune regulation/antigen-independent immune response/idiotypic network/anti-idiotypic Thelper cells)

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Contributed by Niels Kaj Jerne, November 5, 1979

ABSTRACT **Injection in normal mice of IgM antibodies against sheep erythrocytes or dextran, in the form of an immune serum depleted of IgG, induces direct plaque-forming cells of the same specificity as the injected antibodies. The response is 10–70 times higher than the background plaque-forming cell titer of untreated control mice. Nanogram amounts of IgM induce a detectable response, and a ceiling is reached with a few hundred nanograms of monoclonal IgM. The inducing agent is not residual antigen: (*i*) treatment of the injected material and the recipients with dextranase abolishes the immunogenicity of dextran, but not the response to anti-dextran IgM; (*ii*) monoclonal IgM specific for sheep erythrocytes or trinitrophenyl likewise induces plaque-forming cells of the respective specificity, but variant hybridoma products (in which the light chain is that of the myeloma parent) are inactive. In normal mice, IgM-induced antibody responses were observed with antibodies to both thymus-dependent and thymus-independent antigens, but such could not be induced in athymic (*nude*) mice. Because the mechanism underlying this phenomenon would operate also in a normal immune response and, presumably, in the normal dynamic state of the immune system of unstimulated animals, a network regulation among the elements of the immune system itself is implied.**

Classically, the immune system was considered as a set of independent clones of lymphocytes awaiting to be activated or suppressed by antigen, a view that might still be upheld for B cells (1). Alternatively, the network concept (2) envisages connectedness of the sets of combining sites and idiotypes in the B-cell compartment, resulting in mutual regulation of lymphocyte clones in the absence of foreign antigen and blurring the distinction of self and nonself antigens (3). These opposing views bear on the central questions of immunology, and a consensus of opinion is yet to be reached.

It appeared to us that the wealth of observations on enhancement or inhibition of immune responses by antibody might offer further insights when considered in the conceptual framework of an idiotypic network. As a first step we report that foreign antigen is not needed for the induction of specific antibodies. IgM antibodies injected into a normal mouse can induce the production of more antibodies of the same specificity.

MATERIALS AND METHODS

Mice. BALB/c, BALB/c *nu/nu*, C57BL/6J, and C57BL/6 *nu/nu* mice 2–3 months old were obtained from Bomholtgaard (Ry, Denmark) or from the Institut fur Biologisch-Medizinische Forschung AG, Füllinsdorf (BL, Switzerland).

Antibodies. *Conventional antibodies.* Antisera against sheep erythrocytes (SRBC) were raised either by a single injection of 4×10^8 SRBC or by two intraperitoneal injections of the same dose of SRBC 8 weeks apart; the mice were bled 7 days after the last injection. Anti-dextran B512 antisera were raised in C57BL/6 mice by a single intravenous injection of 10 μg of dextran B512. Five days later the animals received 10 units of dextranase (Sigma) and were bled on day 7.

Monoclonal antibodies. Monoclonal antibodies—Sp1/HL with anti-SRBC activity and Sp6/HLK with anti-trinitrophenyl (anti-TNP) activity—were those described by Köhler and Milstein (4). Clones Sp1/HK and Sp6/HK are inactive variants of the above, the specific μ chain being associated with the light chain of the myeloma X63-Ag8. All monoclonal antibodies were in culture supernatants containing 10–15% horse or fetal calf serum. In all experiments, control mice were injected with the same amount of identical, fresh medium. Products of the inactive clones were injected at the same IgM concentration as the specific antibodies.

Preparations of Anti-SRBC and Anti-Dextran B512 Antibodies of Different Class and Subclass. The method of separation on protein A-Sepharose described by Ey *et al.* (5) was used. Anti-SRBC or anti-dextran B512 mouse serum was brought to pH 8.0 by addition of 0.5 M phosphate buffer at pH 8.0, and 2–3 ml was applied to a column of 10 ml (3 g) of protein A-Sepharose CL-4B (Pharmacia, Uppsala), equilibrated with 0.1 M phosphate buffer at pH 8.0. IgG1, IgG2a, and IgG2b fractions were eluted with citrate buffers at pH 6.0, 4.5, and 3.0, respectively. The IgG fractions, as well as the unbound material containing the rest of the serum proteins, were dialyzed and reconcentrated to the original volume of the serum.

The purity of the fractions obtained was checked by double diffusion in agar against class-specific rabbit anti-mouse Ig antibodies (Bionetics, Kensington, MD).

The titer of antibodies was determined by hemagglutination of SRBC, DxB512-coupled donkey erythrocytes (DRBC) (6), or TNP-coupled SRBC or DRBC (7). The tests were performed in Microtiter V plates with 25 μl of serum fractions and 25 μl of 0.5% erythrocyte suspension in saline containing 5% fetal calf serum. After the cells had settled, the direct hemagglutination titer was recorded. The supernatant was then discarded and the pellet was resuspended in 25 μl of anti-Ig antiserum at the appropriate dilution, and the indirect hemagglutinin titer was recorded. The specificity and the suitable dilution of developing antisera were checked on monoclonal anti-SRBC immunoglobulins of known class (4).

Experimental Protocol. Animals were injected intravenously with 0.1 ml of dilutions of serum fractions or culture supernatants containing monoclonal antibodies. Other groups were injected intravenously with 4×10^5 SRBC or 10 μg of dextran B512; control groups received varying amounts of fresh culture medium.

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Abbreviations: B cells, bone-marrow derived lymphocytes; T cells, thymus-derived lymphocytes; PFC, plaque-forming cells; SRBC, sheep erythrocytes; DRBC, donkey erythrocytes; TNP, trinitrophenyl.

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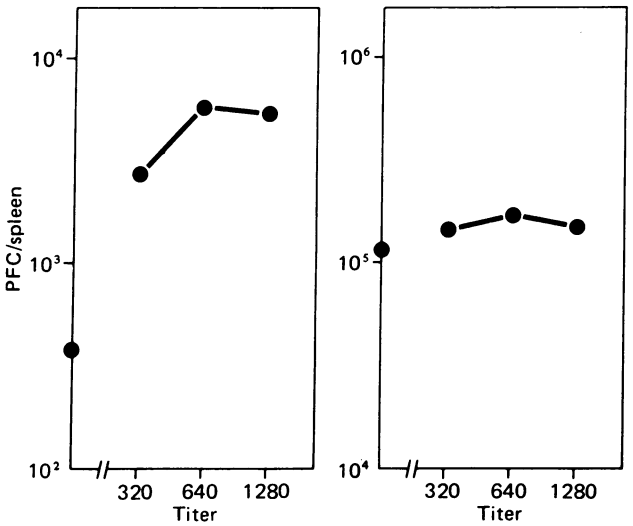

FIG. 1. Anti-SRBC (*Left*) and total IgM (*Right*) splenic PFC in mice 5 days after injection of increasing amounts of syngeneic IgG-depleted anti-SRBC immune serum. Each mouse received 0.1 ml of a serum dilution giving the indicated titer in indirect hemagglutination.

On day 5 or 6 after treatment, spleen cell suspensions were assayed for plaque-forming cells (PFC) to SRBC, DRBC, TNP-DRBC, or dextran B512-DRBC. The same suspensions were also assayed for total number of IgM-secreting cells by a staphylococcal protein A-SRBC plaque assay (8).

RESULTS

We have repeated the experiments and confirmed the results reported by Henry and Jerne (9): The PFC response obtained by injecting low doses of SRBC into mice is greatly enhanced by the addition of small amounts of anti-SRBC immune serum from which IgG antibodies have been removed. We further observed that the spleens of mice receiving this serum fraction alone also responded with increased numbers of PFC to SRBC 5 days later (Fig. 1; Table 1).

PFC to unrelated erythrocytes or the total number of IgM-secreting PFC in the spleen remained unchanged. Preparations of IgG antibodies, separated from the same immune sera that yielded the effective IgM antibodies, had no inducing activity. IgM antibodies were without effect in congenitally athymic (*nude*) mice. This parallels the failure of *nude* mice to respond to limiting doses of SRBC, an antigen known to be largely T-cell dependent (10). Admixture of a small amount of IgM antibody in the plaque assay or incubation of spleen cells with IgM antibody before the plaque assay had no effect on the number of PFC.

Table 1. Anti-SRCB response induced by IgG-depleted anti-SRBC immune sera

Strain	Treatment	Anti-SRBC	Anti-DRBC	Anti-HRBC*	Total IgM
BALB/c	None	339	31		113,581
	SRBC	20,330	92		141,714
	Anti-SRBC IgM	5,736	135		173,195
BALB/c *nu/nu*	None	115		195	169,957
	SRBC	568		154	261,472
	Anti-SRBC IgM	546		179	199,618
C57BL/6J	None	104	30		171,484
	SRBC	7,701	13		73,313
	Anti-SRBC IgM	5,385	26		174,260
C57BL/6J *nu/nu*	None	108	ND		101,895
	SRBC	375	ND		68,423
	Anti-SRBC IgM	442	ND		294,713

The numbers shown are the geometrical means of the numbers of direct PFC per spleen in groups of at least four mice, 5 days after treatment. ND, not done.
* HRBC, horse erythrocytes.

Table 2. Residual dextran in the IgM preparation is not responsible for the response induced by anti-dextran B512 IgM

Treatment	Anti-dextran B512	Anti-DRBC	Total IgM
None	47	28	192,229
B512 (10 μg)	9115	33	191,758
B512 (10 μg) + Dxase (10 units)	25	40	260,872
Anti-dextran B512 IgM	308	31	228,627
Anti-dextran B512 IgM + Dxase (10 units)	573	53	275,979

The numbers shown are the geometrical means of the numbers of direct PFC per spleen in groups of at least four mice, 5 days after treatment. Dxase, dextranase.

The phenomenon observed could have a trivial explanation—namely, the presence of residual antigen in the serum of mice recently immunized. Two types of experiment eliminated this possibility. First, in the case of dextran as antigen, treatment of dextran B512 with dextranase or the simultaneous injection of enzyme and antigen completely abolished the antigenicity of this polysaccharide. Due to chemical degradation of the antigen, the mice behaved as if they had not been exposed to dextran (11, 12). We immunized mice with dextran B512 and treated them with dextranase before collecting the serum (11). Dextranase had no effect on the PFC response induced (Table 2). Thus, dextran cannot be implicated in the effect of the anti-dextran antiserum depleted of IgG antibodies.

Second, the antigen-independence of this phenomenon could be proved by the use of monoclonal antibodies. The injection, into untreated mice, of appropriate dilutions of culture supernatants from a hybridoma that produces anti-SRBC IgM (4) resulted in a vigorous splenic anti-SRBC PFC response. As little as 40 ng of IgM antibody induced a 4-fold increase in anti-SRBC PFC, and the response increased with the amount of IgM injected up to 250–300 ng of antibody (Fig. 2). The corresponding volumes of culture medium failed to induce any discernible response. As also shown in Fig. 2, there was no nonspecific increase in IgM-secreting cells in the spleen at 5 days after injection.

An experiment demonstrating the specificity of monoclonal antibodies is summarized in Table 3. Culture supernatants of the anti-SRBC IgM-producing hybridoma induced an antigen-independent PFC response to SRBC but not to TNP or DRBC, whereas supernatants from an anti-TNP IgM-producing hybridoma induced PFC specific for TNP but not for SRBC. We also tested supernatants from variant cell lines, derived from the original anti-SRBC and anti-TNP hybridomas. The products of these variants had lost antigen-binding specificity after the loss of specific light chains. When injected at the same concentration of μ chains as the specific supernatants, these variant supernatants were ineffective. We conclude that the activity of immune sera depleted of IgG molecules is due to IgM antibodies.

We are much concerned with the magnitude and the re-

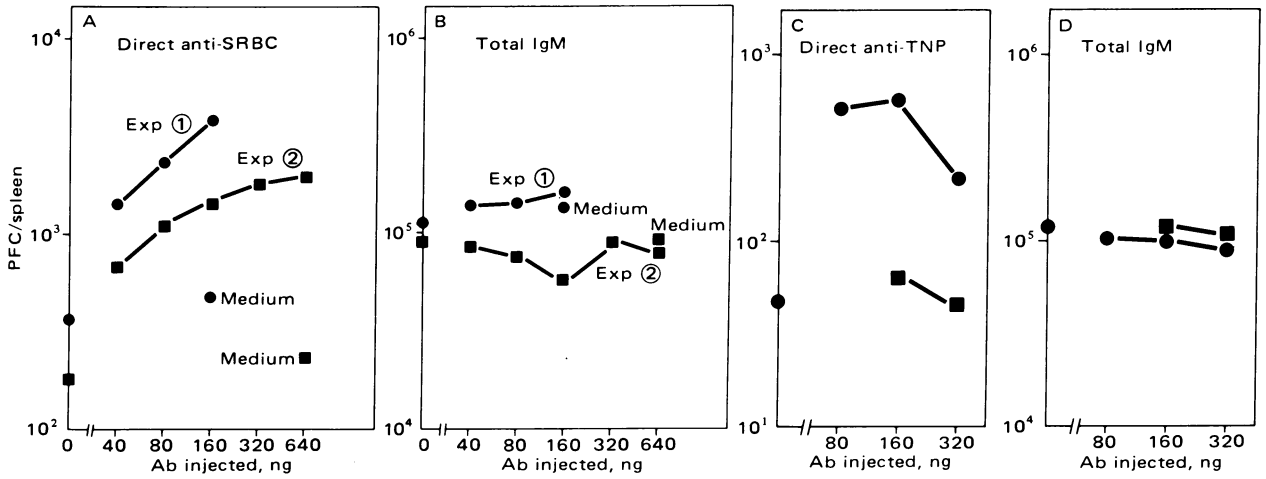

FIG. 2. Specific and total splenic PFC in mice 5 days after injection of increasing amounts of monoclonal IgM antibodies. (*A* and *B*) Anti-SRBC PFC response after administration of anti-SRBC IgM antibody Spl/HL. (*C* and *D*) Anti-TNP PFC response after administration of anti-TNP IgM antibody Sp6/HLK (●) or IgM Sp6/HK variant (■) with unknown antibody specificity.

producibility of these responses. Although the numbers of specific PFC were similar in different individuals treated with the same preparation of IgM antibodies of a given titer, they varied widely among the batches of IgG-depleted immune sera used, from as low as 600 to as high as 60,000 anti-SRBC/spleen (not shown). The responses obtained with monoclonal antibodies remained the same in repeated experiments, reinforcing the impression that variability resides in the antibody preparations and not in the physiological state of the recipients. A point to be stressed is that certain monoclonal IgM preparations we tested induced no specific response. These were the anti-dextran-α-1,3 myeloma protein MOPC 104E and monoclonal anti-phosphorylcholine antibodies isolated by Berek *et al.* (13).

DISCUSSION

No antibody-inducing effect of IgM antibodies could be obtained in T-cell-deprived mice. It could therefore be postulated that the injection of IgM with a given combining site specificity "primes" and expands a population of helper cells (14). These helper cells could either bear idiotypic determinants cross-reactive with the antigen that is recognized by the IgM antibody or, alternatively, they could bear receptors that recognize the idiotopes of the IgM antibody. In the first case the helper cells would be targets for anti-idiotypic recognition by resting B cells of the same paratope specificity as the injected IgM antibodies, and consequently might exert helper functions resulting in the activation of such B cells. In the alternative case, these helper T cells would recognize and activate idiotype-positive B cells, including those bearing the same paratope as the injected IgM. This is the fraction of B cells we have assayed for.

Table 3. Ability of monoclonal antibodies to induce a specific antibody response

Antibody	Specificity	Anti-SRBC	Anti-TNP	Anti-DRBC	Total IgM
None	—	218	48	33	120,164
Spl/HL	Anti-SRBC	3856	65	64	166,857
Spl/HK	Unknown	285	22	30	134,432
Sp6/HLK	Anti-TNP & unknown	185	587	ND	100,842
Sp6/HK	Unknown	161	64	ND	115,854

Spl/HL and Sp6/HLK represent the specific anti-SRBC IgM and anti-TNP IgM. Antibodies of unknown specificity Spl/HK and Sp6/HK consist of the specific μ chain associated with the myeloma X63-Ag8 κ chain. The numbers shown are the geometrical means of the numbers of direct PFC per spleen in groups of at least four mice, 5 days after antibody administration. ND, not done.

These alternatives could be distinguished by determining whether the response induced by injecting IgM antibodies reproduces the paratopes or the idiotopes of the injected molecules. Because the relationship of paratopes and idiotopes is not 1-to-1, the combining site-specific PFCs that we have observed could have been a subset of a larger, idiotype-specific response.

The enhancing effect of IgM antibodies in the presence of antigen may be fundamentally the same as their effect in the absence of antigen. Among the idiotypic universe of natural immunoglobulins, an internal image of any foreign antigen may well be present (2), and lymphocytes might respond to complexes of IgM with molecules bearing this image. Conceivably, helper T cells can respond only to antigen complexed with IgM.

A more general conclusion is that these results support network concepts. Thus, if an antigen-specific response can be induced solely by using components of the immune system itself, it follows that, in its basic economy, this system is autonomous and does not depend on the introduction of antigen to adjust to new dynamic states. A second conclusion relates to the dependence of the effect on helper cells, implying that helper cells with specificities for self idiotypes are functionally competent and available in a normal immune system. This statement, which is supported by a number of other observations (15–20), poses serious questions as to the basis of self–nonself discrimination by helper cells and to the mechanisms of a postulated elimination of self-reactive T cells. As previously discussed (21), it appears that many of the existing helper clones are specific for self Ig idiotypes rather than for nonself antigens. This would be compatible with a normal mechanism of peripheral (post-thymic) expansion of self-reactive T cells exposed to self idiotypes.

We found reproducible responses to the injection of as little as 40 ng of a monoclonal IgM antibody. The responses level off, or may decrease, when more than a few hundred nanograms of IgM is injected. If the responses were induced by a contaminating substance, such as antigen or mitogen, no saturation should be expected at concentrations as low as these. We injected these small amounts of antibody intravenously, without adjuvants. These conditions, under which it is difficult to induce responses to proteins, resemble the internal situation following

an initial primary response. Both normal and germ-free mice show "natural" titers of antibodies against foreign antigens as well as "background" levels of PFC. Consequently, in our view, the normal immune system is continuously maintained in a dynamic state in which its elements (B cells, T cells, immunoglobulin molecules) are engaged in mutual selective regulation.

We have evidence that this applies also to the emergence of selected precursor cells from the bone marrow. When a foreign antigen arrives and is recognized by circulating natural antibodies, an antigen–IgM complex may well be involved in the induction of antibody formation "reproducing" this IgM, as proposed 25 years ago by the natural selection theory of antibody formation (22). After induction of IgM secretion by B cells, this IgM would exert an initial positive feedback leading to the production of more IgM of the same specificity.

We thank Dr. Claudia Henry and Dr. Stephen Fazekas de St. Groth for helpful discussions. The skilled technical help of Ms. Margaretha Tuneskog is gratefully acknowledged.

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