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Regulation of B Lymphocyte Activation by the Fc Portion of Immunoglobulin

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Murine splenic B lymphocytes are induced to proliferate and undergo polyclonal activation in the presence of Fc fragments, AHGG, antigen-antibody complexes, and CH_3 fragments derived from plasmin digestion of human Ig. The unifying feature of the polyclonal antibody response induced by these agents is that in all cases a portion of the constant region of the Ig molecule (ie, Fc region) is present. Fragments of Ig lacking the Fc piece, such as Fab and $F(ab')_2$ were found not to be stimulatory. In addition, a model is proposed to account for the regulatory effects of antigen-antibody complexes on an ongoing humoral immune response.

Key words: Fc fragments, immune complexes, macrophages, polyclonal antibody response

Antigen-antibody complexes [1–11], aggregated gamma globulin [12], and Fc fragments [11, 13–18] are known to be important in both in vivo and in vitro activation and/or regulation of humoral immune responses. These phenomena require the Fc piece, since they are not manifested by Fab or $F(ab')_2$ fragments.

The mechanism(s) involved in activation of bone marrow-derived (B) lymphocytes by Fc fragments, aggregated gamma globulin, and immune complexes may be the same. Murine B lymphocytes were shown to undergo a proliferative response in the presence of Fc fragments derived from mammalian immunoglobulin (Ig) [11, 13–15], and this observation was recently extended to aggregated gamma globulin [12] and immune complexes [11]. Immune complexes formed after the separate addition of antigen and antibody to in vitro cultures resulted in a pronounced proliferative response. Moreover, immune complexes have been shown to be capable of both suppressing [4] and enhancing [3] immune responses, and recently Fc fragments have been shown to enhance weak antibody responses [17, 18], whereas the addition of Fc fragments to strongly responding cultures results in a suppressed response.

Evidence is presented in this report that activation of mouse B lymphocytes by Ig requires the presence of a portion of the constant region of the Ig heavy chain. In addition a model is proposed that describes a role for the Fc region of Ig in regulation of in vivo immune responses.

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MATERIALS AND METHODS

Animals

Male mice of the inbred C57BL/6 strain were obtained from L.C. Strong Laboratories (Del Mar, CA) and Jackson Laboratories (Bar Harbor, ME). All mice were between 8 and 10 weeks of age.

Immunizations

Mice were immunized with 200 μg ovalbumin (OVA) (Miles Laboratories) suspended in Freunds complete adjuvant intraperitoneally (ip). The mice were boosted 30 and 60 days postimmunization with 200 μg OVA in Freunds incomplete adjuvant and were bled 10 days following the last injection. The pooled mouse serum (anti-OVA) was used at a concentration of 0.5% in the polyclonal antibody response.

Preparation of Fc Fragments

A human IgG1 myeloma protein (Fi) was a gift from Dr. Hans L. Spiegelberg, Scripps Clinic and Research Foundation. The IgG1 was purified by ammonium sulfate fractionation, followed by DEAE cellulose chromotography, with 0.01 M phosphate buffer pH 8 used as the eluent.

Fc fragments were obtained by digestion of IgG1 with papain (Sigma Chemical Co.) in the presence of L-cysteine (Sigma) and ethylenediaminetetraacetic acid (EDTA) (J.T. Baker Chemical Co.) for 5 h [19]. Following digestion the material was chromatographed on Sephadex G-100 (Pharmacia Fine Chemicals) to remove any undigested IgG. The Fc and Fab fragments were then separated from each other by DEAE chromatography [20].

Preparation of Heat-Aggregated Human Gamma Globulin

Pooled IgG was obtained as Cohn Fraction II through the courtesy of the American Red Cross National Fractionation Center, with the partial support of National Institutes of Health grant HE-138801, and was purified by DEAE cellulose chromatography, with 0.01 M phosphate buffer pH 8.0 used as the eluent. Human gamma globulin (HGG) was heat aggregated as described previously [21]. The HGG was adjusted to a concentration of 20 mg/ml in 0.01 M phosphate buffer pH 8.0 and maintained at a temperature of 63°C for 25 min. The heated material was then left at 4°C for 24 h prior to collection of the aggregated HGG. The aggregates were precipitated twice with 0.62 M sodium sulfate and dialyzed extensively against phosphate-buffered saline (PBS), 0.001 M phosphate pH 7.2, 0.15 M NaCl before use.

Preparation of $F(ab')_2$ Fragments

$F(ab')_2$ fragments were prepared by digestion of pooled HGG with pepsin (Sigma Chemical Co.) for 18 h [22]. Following digestion, the material was chromatographed on Sephadex G-100 (Pharmacia) to remove any undigested HGG. The $F(ab')_2$ fragments were then heat aggregated as described above.

Preparation of Plasmin-Digested IgG1

Plasmin digests were prepared by incubating the IgG1 myeloma protein for 24 h at 37°C [23]. The digested IgG1 was resolved into two peaks by Sephadex G-150 chromotography. Polyclonal activity was found only in the small molecular weight (CH_3 domain) peak. The optimal concentration was found to be 25 μg/culture.

Polyclonal Antibody Response Assay

For the generation of the polyclonal plaque-forming cell (PFC) response, spleen cells were suspended to a concentration of 6×10^6/ml in RPMI 1640 supplemented as described previously [16]. Duplicate cultures of 6×10^5 cells/0.3 ml were incubated in microtiter plates (3040 Microtest II, Falcon Plastics) at 37°C in 5% CO_2. For the response to immune complexes, 0.5% mouse anti-OVA was substituted for the 0.5% NMS.

Plaque-Forming Cell Response

The response to TNP was assayed by the slide modification of the Jerne plaque assay [24]. Heavily conjugated TNP-SRBC were prepared by the method of Kettman and Dutton [25] and were used as indicator cells to measure the response to TNP. Data are recorded as PFC/10^6 spleen cells ± standard error. Each experiment was performed a minimum of 3 times, and the experiments shown are representative of all the data.

RESULTS

Generation of the Polyclonal Antibody Response

To determine the role of the Fc portion of antibody in the generation of an in vitro polyclonal antibody response, various reagents were assessed for their polyclonal inducing ability. Fc fragments derived from papain digestion of human IgG-induced murine spleen cells to produce a significant polyclonal antibody response (Table I). The polyclonal antibody response produced by Fc fragments was approximately 50% that observed with LPS. Fab fragments and intact IgG were unable to induce a polyclonal antibody response [11, 13, 16].

The fact that intact Ig does not induce a polyclonal response does not preclude the possibility that modification of intact Ig could render it stimulatory. HGG was heat aggregated (AHGG) and assessed for its ability to stimulate murine spleen cells. A significant polyclonal antibody response was produced with AHGG compared to native HGG (157 PFC vs 4 PFC) (Table II). That the presence of the Fc portion of the AHGG was critical for the preparation to be stimulatory was concluded from the observation that aggregated $F(ab')_2$ was unable to activate spleen cells.

Since immune complexes had previously been shown to be able to induce murine splenic B cells to proliferate, it was important to ascertain whether they could function as polyclonal activators as well. Immune complexes were prepared by substituting 0.5% mouse anti-OVA for the 0.5% NMS in culture and adding increased amounts of OVA to the cultures. The results indicate that the maximum polyclonal response was achieved

TABLE I. Fc Fragment Induced Polyclonal Antibody Response

Stimulator	Direct Anti-TNP PFC/10^6 ± SE[a]
–	6 ± 1
Fc fragment[b]	162 ± 25
LPS[c]	345 ± 55

[a]The response was measured on day 3 of culture.

[b]100 μg Fc/culture.

[c]20 μg (E coli 055:B5) LPS/culture.

TABLE II. Aggregated Human Gamma Globulin Induced Polyclonal Antibody Response

Stimulatory	Direct Anti-TNP PFC/10^6 ± SE[a]
–	5 ± 5
HGG[b]	4 ± 3
AHGG[b]	157 ± 13
A(Fab')$_2$[b]	6 ± 1
Fc fragment[b]	171 ± 9

[a]The response was measured on day 3 of culture.
[b]100 μg/culture.

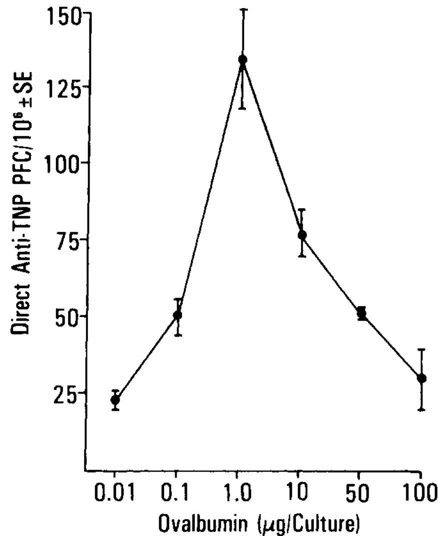

Fig. 1. Increasing amounts of ovalbumin were added to a constant amount of mouse anti-ovalbumin (0.5%). The polyclonal antibody response was measured against TNP-SRBC on day 3 of culture. The direct or IgM plaque-forming response was measured.

when 0.1 μg OVA was added to culture (Fig. 1). It is important to note that as the amount of antibody in mouse serum changes, the peak amount of OVA needed to produce the optimal response also changes (data not shown).

To determine which part of the Fc fragment was needed for the polyclonal response, advantage was taken of the enzyme plasmin, which cleaves between the CH_2 and CH_3 domain, producing Fabc and CH_3 fragments [26]. The small molecular weight plasmin-digested material (ie, CH_3) produced dramatic polyclonal antibody response (19-fold) compared to control cultures (Table III). The polyclonal antibody response generated by plasmin-digested material was always higher than that induced by Fc fragments. Polyclonal

TABLE III. The Ability of Plasmin Digested HGG to Induce a Polyclonal Antibody Response

Stimulator	Direct Anti-TNP PFC/10^6 ± SE[a]
–	36 ± 2
Plasmin digest[b]	678 ± 47
Fc fragment[c]	154 ± 8

[a]The response was measured on day 3 of culture.
[b]25 μg/culture. Sephadex G-150 purified material from plasmin digested HGG.
[c]100 μg/culture.

activiation was never greater than background controls when the large molecular weight plasmin digest was used (data not shown).

DISCUSSION

Murine splenic lymphocytes undergo polyclonal activation in the presence of Fc fragments, AHGG, immune complex, and CH_3 fragments derived from plasmin digestion of human Ig. The unifying feature of the polyclonal antibody response induced by these agents is that in all cases a portion of the constant region of the Ig heavy chain (ie, Fc region) is present. Attempts to induce a polyclonal antibody response with fragments lacking the Fc piece, such as Fab and $F(ab')_2$ resulted in no activation [11, 13].

Fc fragments and AHGG are unique polyclonal activators in that they required the presence of both macrophages and T lymphocytes [12, 16]. The macrophage is responsible for the generation of a mitogenic 14,000 molecular weight Fc subfragment through enzymatic cleavage of the intact Fc fragment or AHGG [12, 15, 16]. These Fc subfragments are responsible for inducing B cells to proliferate, and in the presence of a signal provided by T lymphocytes [16] or soluble factors derived from these cells [27] the B cells differentiate to polyclonal antibody synthesis and secretion. Polyclonal activators such as LPS, dextran sulfate, and purified protein derivative activate B lymphocytes to proliferate and synthesize antibody in the absence of macrophages [8–30]. These results differ from ours in that Fc fragment-induced stimulation has a mandatory requirement for both macrophages and T cells.

The requirement for T lymphocytes has been demonstrated for both the Fc fragment [16] and AHGG [12] induced polyclonal antibody responses. The polyclonal antibody response to immune complexes presumably has parallel cellular requirements, although formal proof is unavailable. That T lymphocytes provide a nonspecific second signal has also been suggested by Parker et al [21]. These authors found that activation of murine B lymphocytes by anti-Ig-coated polyacrylamide beads resulted in proliferation and, in the presence of concanavalin A derived supernate, differentiation to polyclonal antibody production [31].

Other investigators have employed anti-Ig as a probe for studying B lymphocyte activation [31–36]. Parker [32] first reported that purified rabbit anti-mouse Ig covalently linked to polyacrylamide beads could stimulate mouse B lymphocytes to proliferate. Weiner et al [33] observed that the inability of soluble anti-Ig to stimulate mouse B lymphocytes was an age-related phenomenon. The proliferative response to anti-Ig appeared when the mice

reached 5 to 7 months of age. More recently, Sieckmann et al [34] have shown that a soluble purified anti-mouse μ chain had the capacity to activate mouse B lymphocytes to proliferate. Moreover, the proliferative response was independent of macrophages and T lymphocytes [35]. Stimulation by anti-Ig does not appear to be related to the Fc-mediated stimulation described here, because in all reports in the literature where anti-mouse Ig was used it was also demonstrated that $F(ab')_2$ fragments were as effective as the intact molecule [33–35].

It was observed by Sidman and Unanue [36–38] that in the absence of the proper accessory cofactor(s) derived from serum, anti-mouse IgM antibodies were non-mitogenic and in fact were potent suppressors of other mitogenic responses. The cofactor required for the anti-IgM-induced mitogenesis was generated from serum by 2-ME. These authors found that $F(ab')_2$ fragments of anti-IgM were as stimulatory as the intact antibodies [36]. Our results are distinguished from these reports, since AHGG and immune complex induced stimulation are dependent on the Fc piece of Ig.

The cellular events in both the activation of B cells by Fc fragments and the regulatory effect of these fragments on the immune response suggest a model for B cell participation in immunoregulation by antigen-antibody complexes. We have recently observed that Fc fragments have potent adjuvant properties [17, 18]. Fc fragments enhance both the in vivo and in vitro antibody response to SRBC. In addition, the in vitro antibody response to the hapten-protein conjugate TNP-KLH was enhanced. The Fc fragment adjuvant effect was found to be mediated through T cells [18].

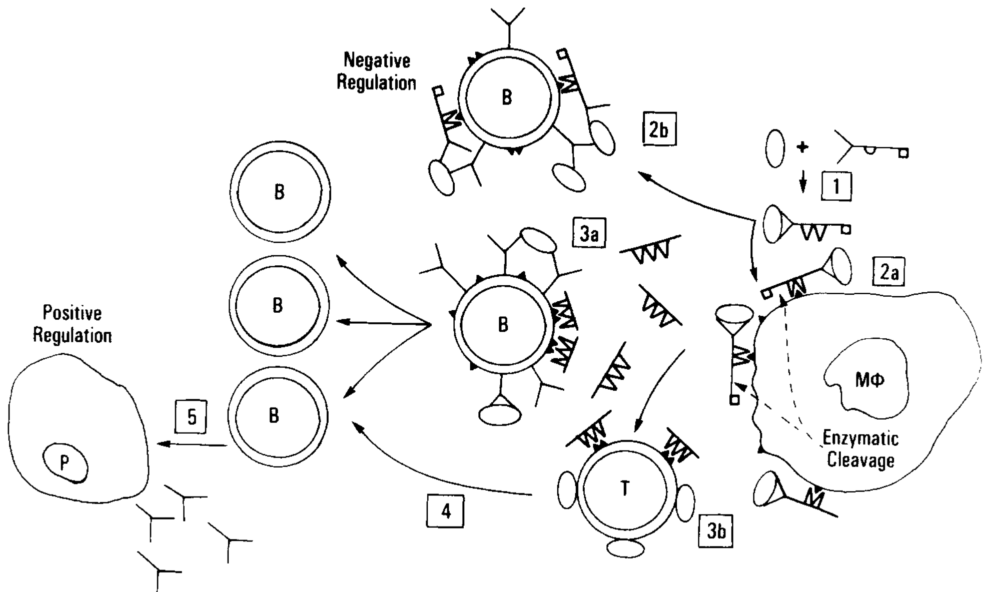

Fig. 2. Proposed immune complex mediated regulation pathway. Antigen-antibody complexes form exposing an Fc attachment site (1); the complex binds to the macrophage Fc receptor, and macrophage enzymes cleave the Fc into an Fc subfragment (2a); the cleavage of the Fc subfragment exposes another binding site, which is recognized by B lymphocyte and T lymphocyte (3b) receptors (3a, b); the binding of the Fc subfragment enhances the antigen-driven response (4,5); when the ratio of antigen to antibody is such that the Fc receptors on the macrophage become saturated, the complexes attach to the B lymphocytes through their Fc receptor and the antigen in the complex crosslinks the Fc receptor and Ig receptor, resulting in a suppression of the response (2b).

Since the cellular events in B cell activation by Fc fragments and antigen-antibody complexes appear to be similar, perhaps the mechanism(s) involved in regulation of B lymphocyte function by Fc fragments may be the same as that involved in regulation by immune complexes. Furthermore, both agents provide positive [3, 11–18] and negative [4, 9–11] signals, which regulate the immune response. The diagram in Figure 2 represents a model for antibody mediated regulation of an immune response. In this model antigen interacts with specific antibody (1), forming an immune complex that results in a conformation change in the antibody molecule potentiating a reactive site in the Fc region. The complex binds either to the surface of macrophages (2a) or B lymphocytes (2b) through an Fc receptor. Since macrophages are responsible for the enzymatic cleavage of Fc and AHGG to mitogenic Fc subfragments [12, 15, 16], binding of the complex to the macrophage surface would facilitate the production of the mitogenic subfragments from these complexes. Cleavage of the subfragment would reveal another binding site, distinct from the site described above, which binds to a receptor an B (3a) and T (3b) lymphocytes. Binding of the subfragment to B and T lymphocytes (3a and 3b) would enhance an ongoing antigen-driven response. If the ratio of antigen to antibody is such that the macrophage receptors become saturated, then the complexes would bind to the B lymphocytes (2b) through an Fc receptor and the antigen in the complex would cross-link the Fc receptor to the Ig receptor, resulting in suppression.

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