REGULATION OF Fc FRAGMENT-INDUCED
MURINE SPLEEN CELL PROLIFERATION*

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The binding of Ig via its Fc portion to various cell populations, through specific Fc receptors, has been shown to mediate a variety of biological functions which include antibody-dependent cell-mediated cytotoxicity and phagocytosis (1, 2). In addition, Fc fragments derived from mammalian Ig-induced murine splenic B lymphocytes to proliferate and synthesize Ig polyclonally (3-5).

The mechanism by which Fc fragments stimulate B lymphocytes is unclear and the exact nature of the mitogenically active material is unknown. Berman et al. (4) observed that an intact disulfide bridge between the two heavy chains, and thus, the intact conformation of the entire Fc fragment, is not critical for the mitogenic activity.

The proliferative response of murine B lymphocytes induced by Fc fragments is dependent upon an adherent macrophage cell population (6). Although adherent cells are required, the interactions between Fc fragments, adherent cells, and B lymphocytes are unknown. Therefore, studies were conducted to determine the role of the adherent cell population in the proliferative response. The studies in this report demonstrate that the interaction of splenic adherent cells with Fc fragments results in the production of a B-cell stimulatory component (14,000 mol wt) derived from Fc fragments.

Materials and Methods

Animals. Male mice of the inbred C57BL/6St strain were obtained from L. C. Strong Laboratories (Del Mar, Calif.). Inbred C57BL/6 nude mice (N_4F_4) were obtained from the Scripps Clinic and Research Foundation breeding colony. All mice were between 8 and 10 wks of age. New Zealand white rabbits were purchased from Rancho Conejo (Vista, Calif.).

Preparation of Fc Fragments. A human IgG_1 myeloma protein (Ff) was a gift from Dr. Hans L. Spiegelberg, Scripps Clinic and Research Foundation. Fc fragments were obtained by digestion of IgG_1 with papain (Sigma Chemical Co., St. Louis, Mo.) as described previously (6).

Preparation of Adherent Cell Supernates. A single cell suspension was prepared by teasing apart the spleens into cold phosphate-buffered saline (PBS),[1] 0.001 M phosphate, pH 7.2, 0.15 M NaCl. The suspension was allowed to stand for 5 min on ice to allow clumps to settle out. The cells were washed twice and resuspended to a concentration of 2×10^7/ml in RPMI-1640

[1] *Abbrevaitions used in this paper:* AD, adherent cell dependent; BSA, bovine serum albumin; FCS, fetal calf serum; LAF, lymphocyte-activating factor; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; SRBC, sheep erythrocytes.

(Flow Laboratories, Inc., Rockville, Md.), supplemented with 5% fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, N. Y.). 1-ml samples were allowed to adhere to tissue culture dishes (3001, Falcon Labware, Div. of Becton, Dickinson, & Co., Oxnard, Calif.) for 1 h in an atmosphere of 5% CO_2 at 37°C. The cells were washed extensively with RPMI-1640 to remove nonadherent cells before the addition of the Fc fragments. Various concentrations of Fc fragments in 2 ml RPMI-1640, supplemented with 2 mM L-glutamine, 1% vitamins, (Grand Island Biological Co.), 100 U penicillin, 100 μg streptomycin (Microbiological Associates, Walkersville, Md.), 5 × 10^{-5} M 2-ME, and 0.5% fresh normal mouse serum were added to the adherent cell layer. The supernatant material was collected and centrifuged at 1,500 g for 10 min in a refrigerated centrifuge. The supernate was then sterilized by filtration through a 0.22-micron filter, stored at −70°C before use and used within 2 wk of preparation.

Chromatography of the Adherent Cell Supernate. The adherent cell supernate was chromatographed on a Sephadex G-50 superfine (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) column (1.2 × 30 cm) with a bed vol of 20 ml. The material was eluted with PBS at a flow rate of 20 ml/h and 0.5-ml samples were collected. The column was calibrated with the following standards, blue dextran (>50,000 mol wt) (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.), deoxyribonuclease (31,000 mol wt) (Sigma Chemical Co., St. Louis, Mo.) and lysozyme (14,300 mol wt) (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.). The molecular weight of the mitogenic material was calculated from a plot of the elution volumes versus the logarithm of the molecular weights of the standards.

Affinity Chromatography. Agarose beads (Bio-gel A50m, Bio-Rad Laboratories, Richmond, Calif.) were activated with cyanogen bromide and either affinity column purified anti-Fc or anti-bovine serum albumin (BSA) were conjugated (7). The Sephadex G-50 derived stimulatory fractions were pooled before affinity column filtration. The pooled material was filtered through the anti-Fc or anti-BSA column, the effluent collected, and concentrated by vacuum dialysis to the original volume. Bound material was removed from the affinity column by elution with 0.1 M acetic acid. The eluted material was immediately neutralized to pH 7 with 1 N NaOH and dialyzed against PBS for 24 h. The material was concentrated to the original volume by vacuum dialysis before use.

Depletion of Macrophages

SEPHADEX G-10 FILTRATION.   Spleen cells were filtered through columns of Sephadex G-10 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.) by the method of Ly and Mishell (8). Sephadex G-10 passage reduced the esterase-positive cells from ∼7-10% to <0.1% (9).

PHAGOCYTOSIS OF IRON FILINGS.   Spleen cells were incubated with iron filings by the method of Lundgren et al. (10). This procedure was found to deplete esterase cells by >90%.

IRRADIATION.   Spleen cells, at a concentration of 5 × 10^6 in RPMI-1640 plus 5% FCS, were exposed to 2,000 R of irradiation from Gamma Cell 40 small animal irradiator (Atomic Energy of Canada Ltd., Ottawa, Canada) delivered at 107 R/min.

Depletion of T Lymphocytes. T-cell-depleted spleen cell populations were prepared by the use of anti-thymus serum as previously described (3, 6).

Depletion of Immunoglobulin Bearing Cells. Ig bearing cells were removed from the spleen cell population by rosetting with glutaraldehyde fixed, F(ab')₂ anti-immunoglobulin coupled sheep erythrocytes (SRBC) by the method of Walker et al. (11). The anti-Ig rosetting reagents were a gift from Dr. Sharyn M. Walker, Scripps Clinic and Research Foundation.

Spleen Cell Proliferation Assay. The method of Berman and Weigle (3), was used for measuring spleen cell proliferation induced by Fc fragments, adherent cell supernates, endotoxin (lipo-polysaccharide, LPS) (*Escherichia coli* 055:B5) (Difco Laboratories, Detroit, Mich.) and concan-avalin A (Miles Laboratories, Inc., Miles Research Products, Elkhart, Ind.). Triplicate cultures of 5 × 10^5 cells/0.2 ml were incubated in flat-bottom microtiter plates (3040 Micro Test II, Falcon Labware, Div. of Becton, Dickinson, & Co.) at 37°C in 5% CO_2. The cultures were pulsed with 1 μCi tritiated thymidine ([³H]TdR) 5 Ci/mM (Amersham Corp., Arlington Heights, Ill.) per 0.05 ml after 2 d of incubation unless otherwise stated, and harvested 18 h later. The results are expressed as mean counts per minute minus the background of triplicate cultures ± standard error. Background counts per minute never exceeded 2,500 cpm. Each experiment was performed a minimum of two times and the experiments are representative of all the data.

TABLE I

Comparison of the Ability of Fc Fragments and Adherent Cell Supernates to Induce Sephadex G-10-filtered Spleen Cells to Proliferate

Material added to the cultures	cpm ± SE
–	2,449 ± 318
Fc*	3,561 ± 179
Fc adherent supernate‡	31,696 ± 294
Normal adherent supernate§	2,566 ± 228

* 50 µg of Fc was added to each culture well.
‡ Adherent cells were incubated with 1 mg of Fc for 1 h before the collection of the supernate. 100 µl were added to each culture well.
§ Adherent cells were incubated with media for 1 h before the collection of the supernate. 100 µl were added to each culture well.

Results

Fc-elicited Adherent Cell Supernatant-induced Spleen Cell Proliferation. Previous studies demonstrated that murine B lymphocytes are induced to proliferate in the presence of Fc fragments (3–5) and the response is dependent upon an adherent cell population (6). To determine whether adherent cells interacted directly with the Fc fragments, adherent cell preparations were incubated with Fc fragments and the cell supernatant assayed for mitogenic activity. This supernate is capable of inducing proliferation in adherent cell-depleted spleen cell cultures (Table I). The Fc proliferation of untreated spleen cell preparations is dependent upon adherent cells and will be referred to as the adherent cell dependent (AD) Fc-induced proliferative response. Proliferation induced by adherent cell supernate occurred in AD cell populations and will be referred to as adherent cell independent (AI) Fc-induced proliferation. The observation that Fc fragments failed to induced AD populations to proliferate indicated that the intact Fc fragments themselves in the adherent cell supernate were not responsible for the observed mitogenic activity.[2] In addition, supernatant material from adherent cells incubated with medium only did not induce proliferation (Table I).

To examine whether the generation of stimulatory supernatants resulted from the in vitro interaction of adherent cells with Fc or if any protein might generate an active supernate, adherent cells were incubated with proteins other than Fc fragments for 1 h and the supernates assayed for stimulatory activity. Fc fragments were found to induce mitogenic supernates, whereas intact IgG_1, Fab fragments, and BSA were unable to induce any activity (Table II).

To determine the maximal activity obtainable from a constant number of adherent cells, 2×10^7 spleen cells were allowed to adhere and then incubated for 1 h with increasing amounts of Fc fragments, the supernates were collected, and assayed on Sephadex G-10 filtered spleen cells. This cell number was chosen to approximate the number of adherent cells found in 5×10^5 untreated spleen cells. Optimal stimulatory activity was obtained from 100 µl of supernate in which 1 mg Fc was incubated with the adherent cells.

To define the optimal time for the generation of the AI Fc response, supernates were collected from 10 min to 48 h after the addition of Fc fragments, and assayed for

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[2] Fc adherent cell supernatant-induced proliferation was assayed on Sephadex G-10-filtered spleen cell populations because intact Fc fragments were unable to induce G-10-filtered cells to proliferate.

Table II

Comparison of Various Proteins for their Ability to Induce Proliferative Adherent Cell Supernates *

Treatment of adherent cells‡	cpm ± SE
Media	1,411 ± 41
1 mg Fc	44,300 ± 1,215
1 mg Fab	<1,000
1 mg IgG₁	<1,000
1 mg BSA	2,464 ± 1,008

* The proliferative response of Sephadex G-10-filtered normal spleen cells to adherent cell supernates.

‡ Adherent cells were incubated with these reagents for 1 h before collection of the supernates. 100 μl were added to each culture well.
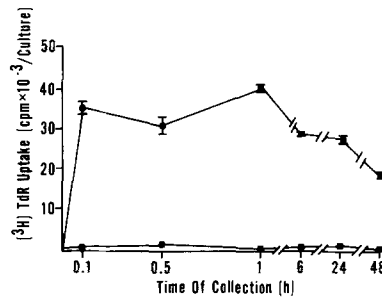

Fig. 1.   Adherent cell supernates were collected from 0.1 to 48 h after the addition of either 1 mg Fc fragments (●) or media (■) (without Fc) to the adherent cells. 100 μl of the supernatant material was added to cultures of Sephadex G-10-filtered spleen cells and proliferation was assayed on day 3.

activity on Sephadex G-10 filtered normal spleen cells (Fig. 1). Supernates collected 10 min after the addition of Fc induced a proliferative response and maximum proliferative activity is obtained from the 1-h supernate. In contrast, supernates from adherent cells that were incubated without Fc fragments contained virtually no activity.

Because stimulatory supernate material was generated after only a 10-min incubation of adherent cells with Fc, the possibility was entertained that either the adherent cells or their products were in some manner modifying the Fc fragments. When Fc fragments or supernate from adherent cells, that were incubated with medium only, were added to Sephadex G-10 filtered cells no proliferation was observed (Table III). However, when a mixture of normal supernate and Fc fragments were incubated together for 1 h and added to Sephadex G-10 filtered cells a significant proliferation resulted. This suggests that the adherent cell supernate is in some way modifying the Fc fragments.

There did not appear to be a genetic restriction in the AI Fc proliferative response because supernate material generated by adherent cells from mouse strains other than C57BL/6St were highly stimulatory (data not shown).

To ascertain the optimum day of the AI Fc induced proliferation, a time-course study was done. The results indicate that maximal proliferation after the addition of the supernate occurred on day 3 of culture (data not shown).

TABLE III

The Ability of Normal Adherent Cell Supernatant Material to Induce Proliferation *

Supernatant source	Added Fc‡	cpm ± SE
Fc adherent§	–	79,941 ± 8,227
Normal adherent‖	–	2,702 ± 288
Normal adherent	+	57,169 ± 1,927
—	+	2,668 ± 551

* The proliferative response of Sephadex G-10 filtered normal spleen cells.

‡ 50 μg of Fc. This concentration was found to give optimal stimulation of normal spleen cells.

§ Adherent cells were incubated with 1 mg Fc for 1 h. 100 μl were added to each culture well.

‖ Adherent cells were incubated with media for 1 h. 100 μl were added to each culture well.

Characterization of the Adherent Cell Supernate. To isolate the stimulatory component(s) in the adherent cell supernate the crude material was subjected to Sephadex G-50 chromatographic separation before assaying for activity. This procedure resolved, by optical density, the supernate into three distinct components, one eluting at approximately the void volume (50,000 mol wt) and the other two <13,400 mol wt (Fig. 2). In contrast, the proliferative activity was found to be maximal at a mol wt of ∼14,000 (Fig. 2) which did not correspond to any of the measurable protein peaks. This result effectively eliminates the possibility of intact Fc fragments directly stimulating the lymphocytes to proliferate.

To test the possibility that the adherent cells were digesting the Fc fragments into smaller molecular weight stimulatory components, the stimulatory material from the chromatographic separation was filtered through an anti-Fc affinity column. The results show that the stimulatory capacity of anti-Fc affinity column effluent was reduced by 60% (Table IV). Moreover, when this material was filtered two times through the anti-Fc column the activity was reduced by 92%. This reduction in stimulatory activity was specific because filtration two times through an anti-BSA column did not significantly reduce the ability of the material to induce spleen cell proliferation. The mitogenic activity was recoverable from the anti-Fc affinity column by elution at low pH (Table V).

Characterization of the Adherent Cell Population Responsible for Mitogenic Subfragment Production. To determine if T lymphocytes were required in the production of the stimulatory supernate, adherent cells from nude mice were assessed. The results indicate that supernate material from nude mice is highly stimulatory for adherent cell-depleted spleen cell preparations. To further establish the lack of T-cell requirement in Fc supernate induced proliferation, normal spleen cell populations were treated with anti-T cell serum plus complement before the generation of supernate. This procedure did not reduce the stimulatory activity of Fc supernate when compared to those produced by untreated splenic adherent cells.

Having established that T cells were not mandatory, the role of cells bearing Ig on their surface in the generation of stimulatory supernatant material were examined. Spleen cells were depleted of Ig-bearing cells by rosetting before being allowed to adhere to plastic. The spleen cell population was found to be depleted of B cells by its
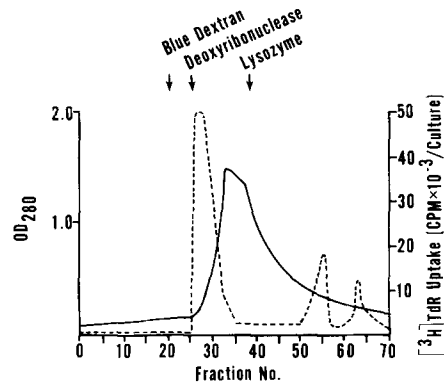
Fig. 2.   Sephadex G-50 superfine chromatographic separation of Fc-adherent cell supernate. Each column fraction was assayed for protein content by OD and for mitogenic activity. The mol wt of the standards were: blue dextran (<50,000), deoxyribonuclease (31,000), and lysozyme (14,300). OD_{280} (- - -), mitogenic activity (——).

TABLE IV

The Ability of the Anti-Fc Affinity Column to Remove the Mitogenic Activity from the Adherent Cell Supernate

Supernatant source	Treatment	cpm ± SE	Reduction*
			%
Fc adherent‡	None	39,813 + 822	—
Fc adherent	Anti-Fc§ (1×)	12,393 ± 548	69
	Anti-Fc (2×)	3,229 ± 781	92
Fc adherent	Anti-BSA‖ (1×)	37,166 ± 1,089	7
	Anti-BSA (2×)	44,119 ± 3,334	0

* (1-[affinity column filtered/nonfiltered]) × 100.
‡ The 14,000-mol wt fraction from the Sephadex G-50 chromatographic separation of Fc adherent cell supernate.
§ 1×—filtered once, 2×—filtered twice. 100 μl were added to each culture well. The material was filtered through an anti-Fc affinity column before use.
‖ The material was filtered through an anti-BSA affinity column before use.

TABLE V

Recovery of the Mitogenic Material from the Anti-Fc Affinity Column

Supernatant source	Treatment	cpm ± SE
Fc adherent*	None	44,510 ± 3,270
Fc adherent	Anti-Fc column effluent (2×)‡	4,714 ± 992
Fc adherent	Anti-Fc column eluate	30,214 ± 1,838

* The 14,000-mol wt fraction from the Sephadex G-10 chromatographic separation of Fc adherent cell supernate.
‡ 2×—filtered twice. 100 μl was added to each culture well.

inability to respond to LPS, whereas a normal response to Con A was generated. Supernatant material generated from adherent spleen cell populations depleted of Ig-bearing cells are as stimulatory as untreated adherent cell preparations.

To determine whether the adherent cell responsible for the cleavage of the Fc

The Ability of T-lymphocyte-depleted Cell Populations to Proliferate

Spleen cell source	Stimulator	cpm ± SE
Nude*	LPS‡	146,157 ± 5,929
Nude	Con A§	1,685 ± 362
Nude	Fc‖	33,647 ± 1,303
Nude G-10 filtered¶	Fc	<1,000
Nude G-10 filtered	Fc-adherent supernate**	20,172 ± 960
Nude G-10 filtered	adherent supernate‡‡	2,315 ± 1,210

* Untreated C57BL/6 nude spleen cells.

‡ 20 µg/culture.

§ 0.2 µg/culture.

‖ 50 µg/culture.

¶ The spleen cells were filtered through Sephadex G-10 columns prior to culture.

** Supernatant fluid from splenic adherent cells incubated with 1 mg Fc for 1 h.

‡‡ Supernatant fluid from splenic adherent cells incubated with media for 1 h.

fragments and the subsequent generation of mitogenic subfragments was a macrophage, the following experiments were conducted. Filtration of the spleen cells through columns of Sephadex G-10, before adherence, was found to significantly reduce the cell population's ability to generate mitogenic supernates (32,832 vs. 7,587). When the cell preparation was depleted of phagocytic cells by ingestion of iron filings before plastic adherence, there was little demonstrable mitogenic supernatant produce (38,400 vs. 2,703). Finally, irradiation (2,000 R) of the spleen cell population did not affect the ability of the adherent cells to produce mitogenic supernates (43,676 vs. 46,078). Thus, the adherent cell responsible is Ig negative and not a T cell.

Characterization of the Proliferating Cell Population. To determine whether the cell population which responds to the adherent cell supernate was composed of T cells, spleen cells from nude mice were assessed. Untreated nude spleen cells respond to Fc fragments, as previously observed (Table VI). Sephadex G-10 filtration of the nude spleen cells before the addition of the Fc fragments reduces the response from 33,647 to <1,000 cpm. When the Sephadex G-10 filtered cells were incubated with the adherent cell supernate prepared with Fc fragments, a significant proliferative response occurred (Table VI).

The role of Ig-bearing cells in the proliferative response was measured by depleting the spleen cell population of Ig-bearing cells by rosetting with anti-Ig before stimulation. The removal of Ig-bearing cells reduced the AI Fc proliferative response from 29,724 to 3,364 cpm (89%) indicating that an Ig-bearing cell was the major cell type responsible for the proliferative event. To determine the effectiveness of this procedure to selectively deplete Ig-bearing cells, the depleted population was compared with its normal counterpart for the ability to respond to LPS and Con A. The response to LPS was reduced by 93% whereas the response to Con A was reduced by only 17%. These results indicate that the population which is proliferating is primarily composed of B lymphocytes.

Discussion

Murine splenic lymphocytes proliferate in response to the supernate derived from Fc fragment-pulsed splenic adherent cells. This proliferative event is not a result of

intact Fc fragments in the supernate because (a) the molecular weight of the mitogenic factor is ~14,000 and that of Fc is 50,000; (b) and the supernatant-induced proliferative response is adherent cell independent whereas Fc proliferation is adherent cell dependent. That the stimulatory supernates result from the interaction of Fc fragments with adherent cells and not with other proteins (IgG$_1$, Fab, or BSA) is deduced from the observation that incubation of other proteins with adherent cells does not result in the production of stimulatory supernates.

The adherent cell responsible for the cleavage of the Fc fragments is a macrophage because it is esterase positive, Ig negative, removed by Sephadex G-10 filtration, phagocytic, and irradiation resistant. An explanation which could account for the rapid production of the mitogenic supernate is that the Fc fragments are enzymatically digested into lower mw stimulatory products by the adherent cells. Proteolytic enzymes secreted by adherent cells could cleave the Fc fragments into smaller subfragments which then directly stimulate lymphocytes to proliferate. This possibility is favored because the stimulatory material appears to be a product of Fc, in that it binds to and can be recovered from an anti-Fc affinity column. Binding of the Fc fragments to the adherent cells is not a mandatory requirement for the generation of mitogenic subfragments because this material can be generated with adherent cell supernate.

The observation that the macrophage appears to cleave the Fc fragments into smaller biologically active subfragments is reminiscent of the work demonstrating that macrophages and macrophage-derived soluble factors act upon the antigen instead of the reactive lymphocytes (12–15). It was originally reported by Hoffmann and Dutton (12) that supernatant material derived from macrophages would restore the antibody response of macrophage-depleted spleen cell cultures. Work by other investigators has shown that the macrophage supernate acts by breaking down or solubilizing SRBC, thus creating antigenic fragments which stimulate lymphocytes directly (13–15). Evidence is provided in the literature to support the contention that polypeptides derived from enzymatic cleavage of Ig are able to mediate certain biological functions. Functions such as prevention of IgE-mediated mast cell degranulation (16), activation of macrophages and polymorphonuclear leukocytes (17, 18), and enhancement of the macrophage-dependent immune education of T lymphocytes (19).

Although macrophages secrete a wide variety of biologically active factors, the mitogenic material in the supernatant is derived from Fc fragments. Preliminary characterization of the mitogenic material indicates that it is ~14,000 mol wt in size. Macrophages have been shown to secrete factors, most notably lymphocyte-activating factor (LAF) (10,000–20,000 mol wt), which modulate lymphocyte function (20–23). The mitogenic material in the system described here is not LAF because LAF has been shown to act primarily on T cells (review in reference 23) and T cells do not appear to play a role in our system. Second, the mitogenic material binds to an anti-Fc affinity column indicating that it is derived from the Fc fragments. Furthermore, Hoffmann et al. (24) found that LPS-stimulated macrophages produce a soluble mediator that substitutes for helper T cells and induces a phenotypic differentiation of B cells. This factor was characterized to have a mol wt of ~15,000. Although the Fc subfragment described here is clearly mitogenic for B cells we cannot rule out the possibility that it is complexed with some macrophage product (e.g., histocompatibility product). The possibility of a complex appears unlikely, however, because of the

size of the subfragment and the fact that it retains antigenicity. Moreover, there appears to be no genetic restriction in lymphocyte stimulation by the mitogenic material.

The spleen cell population responsible for the proliferative event is composed of B lymphocytes. The proliferating cell bears surface Ig, is present in nude mice, and is not depleted by Sephadex G-10 filtration.

The regulation of B lymphocyte activation by Fc fragments, aggregated Ig, and immune complexes may be mediated through similar pathways. Furthermore, it was shown that both aggregated Ig and antigen-antibody complexes are capable of inducing B cells to proliferate (5). The addition of aggregated IgG but not untreated IgG$_1$, to the macrophage cultures results in a highly stimulatory macrophage supernate (E. Morgan and W. O. Weigle. Manuscript in preparation.). It may be that either the digestion of IgG$_1$ with papain or aggregation by heat or specific antigen induces some change in the tertiary structure of the Fc portion of the molecule exposing a binding site for the macrophage enzyme.

Regulation of the in vivo immune response by immune complexes could occur by macrophage modulation of the Fc portion of antibody in the complex. Macrophages do bear Fc receptors on their surface (2), therefore immune complexes could be localized on their surfaces. This localization would then allow the macrophage proteolytic enzymes to digest the antibody producing the regulatory subfragment. The subfragment would then be released in close proximity to the B cells and cause an increase or decrease in the response depending upon the activation state of the B cells. This hypothesis is supported by the findings that Fc fragments suppress as well as enhance B-cell responses (5). Immune complexes are known to have the capacity of both enhancing (25) and suppressing (26) the immune response as well. Experiments are currently in progress to determine whether such a mechanism could account for the observed regulation of the immune response by immune complexes.

Summary

Murine splenic lymphocytes proliferate in response to supernatant material derived from Fc fragment-pulsed splenic adherent cells. The stimulatory supernatant results from the interaction of Fc fragments with adherent cells or adherent cell supernate. Isolation of the stimulatory material in the supernate by Sephadex chromatography revealed that the mitogenic component was a cleavage product of Fc with a mol wt of ~14,000.

The spleen cell type responsible for the generation of mitogenic Fc subfragments appears to be a macrophage. Unstimulated macrophages release an active supernate without being exposed to Fc fragments. The supernate of unstimulated macrophages apparently contain an enzyme which is capable of cleaving Fc fragments into the 14,000-mol wt mitogenic molecules.

The spleen cell population induced to proliferate in response to the adherent cell supernate is present in T-cell depleted and Sephadex G-10 filtered cell preparations. Depletion of cells bearing immunoglobulin on their surfaces results in a reduced proliferative response to the mitogenic supernatant material indicating that it is probably a B cell.

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