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Research Article

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Dysfunctions of Pokeweed Mitogen-stimulated T and B Lymphocyte Responses Induced by Gammaglobulin Therapy

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ABSTRACT Lymphocytes obtained from nonimmuno deficient children treated with commercially available preparations of gammaglobulin failed to proliferate and to mature into plasma cells in vitro after stimulation with pokeweed mitogen. The influence of the treatment on lymphocyte functions varied according to the cell population considered. A T helper cell activity was detected in these patients but only in the cell subset bearing receptors for IgG after irradiation. T lymphocytes exerted a suppressive effect that disappeared after irradiation or incubation at 37°C. The suppressive cells were found among E rosette-forming cells depleted of leukocytes bearing receptors for IgG. Their suppressive effect was expressed only in the presence of normal radioresistant T lymphocytes that did not bear Fc receptors for IgG. Similar dysfunctions could be induced in vitro by incubation of normal T and B lymphocytes with gammaglobulin preparations. Because F(ab)'2 fragments or deaggregated preparations of gammaglobulin failed to activate T suppressor lymphocytes, this activation was likely triggered by attachment of Fc portion of denatured IgG to the corresponding membrane receptor. This activation step was prostaglandin E_2-dependent, suggesting that activated monocytes were involved in the activation process. B lymphocyte responses appeared directly inhibited by attachment of denatured gammaglobulin on membrane Fc receptor. Our observations suggest that immunological effects of gammaglobulin therapy are not limited to antibody transfer, since it also induces subtle modifications of in vitro pokeweed mitogen-stimulated T and B cell responses. These modifications must be considered in interpreting results obtained in immunodeficient patients investigated under gammaglobulin therapy.

INTRODUCTION

Immune responses are under a regulatory control system in which helper and suppressor mechanisms are involved. Besides regulatory processes of the immune response to a single antigen, several animal and human studies have demonstrated leukocyte populations able to suppress antigen or mitogen-induced T and B cell functions (1–8). Suppressor functions have been ascribed predominantly to T lymphocytes activated either by fixation of immune complexes on Fc receptors for IgG (Fcγ) (5) or by mitogens, such as concanavalin A (Con A) (4, 7–10). Other suppressor leukocytes have been described including B lymphocytes (11) and monocytes (12–15) acting through the release of suppressor mediators, especially prostaglandin E_2 (PGE_2)[1] (13–16). Cell-to-cell interactions between subpopulations of T cells (2, 17) or T lymphocytes and monocytes (18) also play an important role in the expression of suppressor activities. The physiological relevance of these various immunoregulatory systems remains undetermined. In addition to these numerous studies showing that expression of suppressor activities requires an in vitro activation, naturally occurring suppressor lymphocytes have been shown to control the level of T and B immune responses (19–23) at such times as the neonatal period (24–26), late pregnancy (26), and in normal nonresponder subjects (27). The

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[1] *Abbreviations used in this paper*: E-RFC, sheep erythrocytes forming rosettes; γG, gammaglobulin; HTLA, human T leukocyte antigen; ICC, immunoglobulin-containing cells; NWC, nylon wool columns; PGE_2, prostaglandin E_2; PWM, pokeweed mitogen; sIg, surface immunoglobulins; TS, T suppressor.

generation of plasma cells during pokeweed mitogen (PWM)-stimulated leukocyte cultures allows the study of the balance between positive and negative regulatory functions. This technique appears to give reproducible results in autologous as well as in allogeneic systems.

As it was suggested that immunoglobulin may reduce the level of the humoral response (28), and because an excess of suppressor activity was described in hypogammaglobulinemic patients receiving gammaglobulin (γG) therapy (29, 30), we have investigated the influence of γG injections in children without any detectable immune deficiency on the regulatory mechanism of the PWM system. We have found that γG injections induce an inhibition of B lymphocytes and an activation of T suppressor (TS) lymphocytes that can inhibit the proliferation of T and B lymphocytes and the maturation of B lymphocytes. Furthermore, γG injections provoked the lack of the helper function normally expressed by lymphocytes that do not bear Fcγ receptors. In an attempt to define the mechanisms involved in the inhibition of B and activation of TS lymphocytes, we developed experiments using normal leukocytes incubated with γG preparation.

METHODS

Blood was collected from 12 children (aged 4 to 12 yr) who had no detectable abnormality of immune functions and who received γG therapy because of mild but repeated infections of the respiratory tract. Humoral and cellular immune functions were investigated in all children before therapy. Antibody formation and serum immunoglobulin levels were within normal ranges except for three who had an increased serum IgE level (230 to 850 IU/ml, $n = 60 \pm 40$ IU/ml as tested by radioimmunoassay). In vivo delayed skin reactions to antigens (tuberculin, purified protein derivative, and Candidin [CD]) and in vitro proliferative responses to mitogens (phytohemagglutinin, Difco Laboratories, Detroit, Mich.), Con A (Miles Laboratories, Yeda, Israel), PWM (Gibco Laboratories, Glasgow, Scotland) or antigens (tetanus toxoid, purified protein derivative, and CD) performed as previously described (31) were comparable to controls.

10 children were treated with plasmatic γG obtained from blood transfusion center (Centre National de Transfusion Sanguine, France), prepared by fractionation of plasmatic human γG using ethanol fractionation (32), and adjusted to a final concentration of 165 mg/ml in a solution containing 22.5 mg/ml glycine and 0.1 mg/ml sodium mercurothiolate. Electrophoretic and immunoelectrophoretic studies showed that γG preparations contained a large proportion of IgG and traces of IgA, IgM, and 2% albumin. As measured by radial immunodiffusion, IgG represented 155 mg/ml (94%); IgA, 1.5 mg/ml; IgM, 0.5 mg/ml. Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) gel filtration pattern of the γG preparation used for therapy showed the presence of ~30% of aggregates (>150,000 mol wt) (Fig. 1). No antibody against histocompatibility antigens (A, B, and DR) was detected using 10–80 mg/ml γG preparation in a microcytotoxicity test (33).

Two other children were treated with placental γG (Institut Mérieux, Lyon, France). The γG preparation was eluted from placenta at pH 2.5, then fractionated by ethanol precipitation (32), and adjusted at 165 mg/ml. It also contained aggregates (Fig. 1).

The γG preparation was injected intramuscularly every 15 d at the dosage of 80 mg/kg, resulting in a reduction of the frequency of infections. All children were investigated prior to, during (48 h after the third or the fourth injection), and after (1–8 mo) cessation of therapy. Among tested functions, the only change observed was a normalization of the serum hyper-IgE. Under therapy, neither antiimmunoglobulin antibodies nor immune complexes were detected by a hemagglutination technique (34) or by polyethyleneglycol method (35), respectively. Apart from profound decreases in the PWM-induced responses, no other changes of cell-mediated immunity including in vitro cellular responses and in vivo delayed skin reactions to antigens were observed. Although a longitudinal study of antibody formation was not performed, we noticed a return to normal of serum IgE levels, whereas other serum immunoglobulin classes remained unchanged.

A control group consisting of healthy adult donors or age-matched children was studied in parallel. All blood specimens from children were obtained following informed consent by the parents.

Leukocyte markers. Blood was collected in preservative-free heparin (100 U/ml). After dilution 1:3 in Hanks' buffer solution (Gibco Laboratories) mononuclear cells were isolated on Ficoll-Hypaque (Pharmacia Fine Chemicals) density gradient and washed twice in medium. Sheep erythrocytes forming rosettes (E-RFC) were counted as previously described (36). A heteroantiserum specific for human T leukocyte antigen (HTLA) (Institut Mérieux) was also used in a microcytotoxicity test, and results were expressed after substraction of the background. Surface immunoglobulins (sIg) were detected by membrane immunofluorescence using fluorochrome-labeled monospecific F(ab)'2 fragments against human μ-, δ-, γ-, and α-chains and anti-F(ab) IgG (Nordic Laboratories, Tilburg, Netherlands) (37). In order to detect actual products of B lymphocytes, cells were preincubated 30

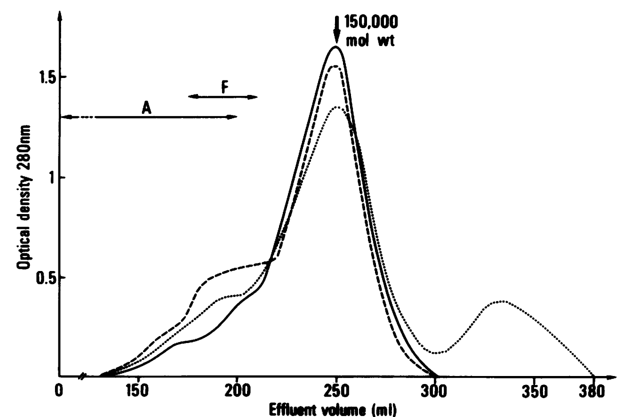

FIGURE 1 Elution patterns from Sephadex G-200 of plasmatic (-----) and placental (·····) γG preparation and purified human IgG (——): F, elution zone of ferritin (mol wt, 450,000), A, elution zone of heat-aggregated human IgG. γG preparation appears to contain ~30% aggregates (mol wt > 150,000). Placental γG preparation was contaminated by a heterogeneous polypeptide (~50,000 mol wt) as defined by polyacrylamide gel (45); this constituant was precipitated by anti-IgG antiserum in double immunodiffusion and was found not to be suppressive in the PWM system.

min at 37°C or treated with trypsin (ICN Pharmaceuticals Inc., Cleveland, Ohio) (2 mg/ml), then washed and incubated for 7 h before sIg studies were performed. Detection of C_3b and Fc receptors was performed by EAC (erythrocytes antibody complement) rosetting and fixation of heat-aggregated rabbit IgG revealed by a fluorescent sheep antiserum anti-rabbit IgG (38). Ia-antigen was revealed by an anti-Ia antiserum raised in rabbit (kindly donated by Dr. P. Niaudet, Institut National de la Santé et de la Recherche Medicale, Paris) and prepared according to a technique previously described (39).

Determination of E-RFC-bearing Fc receptors for IgG (EAγ^{+} cells) was done as described by Moretta et al. (5). Detection of endogenous peroxidase was done as described by Preudhomme and Flandrin (40). Histochemical staining for α-naphthyl esterase was used to define subpopulations of human blood T lymphocytes and monocytes, as described by Horwitz et al. (41).

Cell separation. E-RFC positive or E(+) and E-RFC negative or E(−) leukocytes were obtained as already described (26). After erythrocyte lysis, preparations from controls and children who received γG-E(+) contained 85.4±6.9% HTLA(+), 87.8±7.5% E-RFC, 0.5±0.9% sIg(+) and 3.7±2.3% peroxidase positive cells. In E(−) cell preparations of both origins, some T cells (9.6±3.3% HTLA[+] and 5.5±2.8% E-RFC), 45.4±6.7% sIg(+) and 28.7±12.1% peroxidase positive cells were enumerated. A further step of E(+) cell separation was performed by rosetting with IgG-sensitized ox erythrocytes by a previously reported method (26). EAγ(+) cell preparations contained 62.2±6.8% HTLA(+), 58.5±6.5% E-RFC, and 59.9±6.5% cells formed again EAγ(+) rosettes. 13.0±6.1% ox erythrocytes phagocytizing cells and 4.1±2.9% peroxidase positive leukocytes were also detected. EAγ(−) cell preparations contained 85.8±8.5% HTLA(+), 86.1±4.7% E-RFC, few peroxidase positive cells (1.5±0.5%) and very few cells able to form EAγ(+) rosettes (≤0.5%).

Adherent and nonadherent cells were obtained by incubation of 150–500 × 10^3 E(−) cell preparations at 37°C in plastic microwells (Falcon 3040, Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.). After 1 h, nonadherent cells were removed and adherent cells were washed thoroughly several times with Hanks' solution. About 20% of cells adhere on plastic and 90.5±6.2% were monocytes as judged by latex particle phagocytosis. Nonadherent cells were further depleted of monocytes by two successive incubations on plastic at 37°C. In some experiments, indomethacin (Sigma Chemical Co., St. Louis, Mo.), at a final concentration of 0.51 μM, or anti-PGE_2, or antithromboxane B_2 (TxB_2) antisera raised in rabbit (42), at a final concentration 1:100, were added.

Various pretreatments of cell preparations were performed in particular experiments: pretreatment of E(−) preparations either by trypsin or by a cytotoxic anti-HTLA in the presence of complement, filtration of E(+) or EAγ(−) cells through nylon wool columns (NWC) as described by Julius et al. (43) (0.5±0.9% peroxidase positive cells were enumerated), incubation of E(+), EAγ(−), or EAγ(+) leukocytes during 24 h at 37°C in RPMI 1640 medium (Gibco Laboratories) containing 20% decomplemented human AB+ serum or irradiation of E(+), EAγ(+), or EAγ(−) cells at 2,000 rad in a gamma cell irradiator.

Elsewhere, before being cultured, control E(−), E(+), or EAγ(−) leukocytes were incubated with extensively dialyzed plasmatic (batches 77 D277 A and 77 D 853 F) or placental (batch U 0 143 I) γG preparations (0.07–1.5 mg/ml).

In other experiments, control E(+) or EAγ(−) cell populations were incubated with the same concentrations of aggregated or deaggregated γG(separated by gel filtration on Sephadex G-200) or of F(ab)′2 fragments of plasmatic γG(prepared by peptic digestion (pepsin, Sigma Chemical Co.) 20 h, 37°C, followed by gel filtration, according to the technique of Natvig and Turner [44]). Purity of F(ab)′2 fragments was verified on sodium dodecyl sulfate polyacrylamide gel (45). Finally, control E(+) cell preparations were incubated with the same concentrations of plasmatic or placental γG previously absorbed (vol/vol) with a pool of total (times three) and E(−) (times two) normal leukocytes before testing for suppressive activity.

In all experiments, cell viability was >90% as judged by trypan blue exclusion.

Cell cultures. PWM-stimulated cultures were performed as previously described (26). Cultured cells, harvested at day 7, were counted and stained for the presence of intracytoplasmic immunoglobulin with fluorescent monospecific anti-heavy chain (N. L. Cappel Laboratories, Inc., Cochranville, Pa.) or polyvalent anti-human immunoglobulin (Meloy Laboratories, Inc., Springfield, Va.) antisera. The proportion of immunoglobulin-bearing and immunoglobulin-containing cells (ICC, or plasma cells) was determined by examination of 500–1,000 cells. Proliferative responses, measured by incorporation of tritiated thymidine (2 μCi/ml, sp act 1 Ci/mM) added during the last 18 h of the culture were expressed in Δ-counts per minute, after subtraction of the background.

Statistical analysis. Data are expressed as the mean value ±1 SD. The level of significance (P) was obtained using Student's t test.

RESULTS

Effect of γG injections on blood lymphocyte markers. Absolute number and expression of surface markers of T and B lymphocytes remained unchanged during γG therapy. Double staining experiments showed, however, that 25% of μ- and δ-bearing B lymphocytes were also detected by F(ab)′2 fragments of anti-γ-chain antibodies. γ-chains that cocapped with Ia antigens were thought unlikely to be B lymphocyte products as they did not reappear after an in vitro trypsin treatment.

Reduced capability of γG-treated children leukocytes to proliferate and to mature into ICC during a PWM-stimulated culture. Leukocytes from 10 children before therapy were capable of proliferation and maturation into ICC in the presence of PWM, comparable to adult or age-matched normal children. In contrast, leukocytes from the same children receiving plasmatic γG injections proliferated less well than normal leukocytes ($P < 0.01$) and demonstrated reduced plasma cell maturation ($P < 0.001$) (Table I). Studies of membrane markers indicated that the reduction of proliferation occurred in both T and B cell populations and showed that, although δ-chain determinants normally disappeared, γ- and α-bearing lymphocytes were strongly diminished. The same abnormalities, absent before therapy, appeared in two children receiving γG of placental origin.

Longitudinal studies performed in six children indicated that this failure persisted several months (up to 5 mo) after cessation of γG therapy. After restoration of PWM-induced responses we observed, in three chil-

Table I

PWM-induced Proliferation and Maturation into Plasma Cells of Lymphocytes from Control or from Children prior to or during γG Therapy

Source of cultured leukocytes	No. of subjects tested	Δ cpm × 10³	Absolute number of recovered cells × 10³±1 SD: Total leukocytes	Plasma cells containing: IgM	IgG	IgA	IgD	Cells bearing: IgM	IgG	IgA	IgD
Control	35	25.9±6.6	150.5±62.0*	11.6±7.8	9.6±5.8	9.6±6.9	<0.1	10.7±5.2	8.0±2.1	5.9±0.5	<0.1
Children before γG therapy	10‡	39.0±16.9	209.0±19.2	11.8±4.3	8.4±3.7	5.8±2.1	<0.1	11.8±6.8	7.4±3.9	6.7±0.7	<0.1
Children receiving plasmatic γG	10‡	11.7±8.2	91.5±20.5*	0.3±0.3	0.3±0.3	<0.1	<0.1	5.4±1.5	1.8±2.1	0.8±0.2	<0.1

Cultures were performed with 200 × 10³ leukocytes in presence of PWM for 7 d.

Detection of membrane determinants (HTLA or sIg) performed at the end of cultures * indicated that the reduction of proliferation affected both T and B cells since respective percentages (44.5±10.4 and 10.5±2.5%) were not different from control cultures (42.3±2.8 and 15.7±3.2%).

‡ Two additional children were tested prior to and during therapy with γG of placental origin. Results were very similar to those obtained with the 10 children receiving plasmatic γG.

dren tested, that reintroduction of γG injections provoked the same failure (Table II).

Inability of B lymphocytes from γG-receiving children to mature into ICC with the help of normal T cells. B lymphocytes present in E(−) populations isolated from γG-receiving children proliferated poorly ($P < 0.001$) and did not mature into ICC when co-cultured with control E(+) leukocytes ($P < 0.001$) (or their own E[+] leukocytes) (Table III). B lymphocytes remained unable to mature after 7 h incubation in culture medium alone or after pretreatment with trypsin or after depletion of a few T cells that can contaminate E(−) preparations by a cytotoxic anti-HTLA antiserum, while normal B lymphocytes were not affected under such conditions (data not shown). On the other hand,

Table II

Longitudinal Study of PWM-induced Maturation into Plasma Cells of Lymphocytes from Children prior to, during, and after (1–6 mo) Cessation of Treatment with Plasmatic γG

Children	No. of recovered plasma cells × 10³: Prior to γG therapy	During γG therapy	After cessation of γG therapy: 1 mo	2–5 mo	6 mo	During a second cure of γG therapy
1	10.5	0.2	NT	1.8	27.8	NT
2	15.0	0.1	NT	0.3	18.9	NT
3	18.2	0.2	0.1	NT	24.9	0.1
4	12.0	0.1	0.8	7.2	12.2	0.2
5	16.8	0.1	0.1	14.9	NT	0.1
6	5.6	<0.1	NT	4.8	NT	NT

PWM-stimulated cultures were performed using 200 × 10³ leukocytes isolated from the same children before any therapy, during therapy with plasmatic γG, 1–6 mo after cessation of treatment and during a second cure of plasmatic γG. NT, not tested.

several experiments were performed to determine whether or not monocytes were involved in the failure of B cell maturation. Depletion of adherent cells or their replacement by control adherent cells had no significant influence on the generation of PWM-induced plasma cells. Addition of indomethacin, known to switch off a part of monocyte suppressor activity (46), or of an anti-PGE_2 antiserum, did not restore the B lymphocyte maturation.

Helper effect of T lymphocytes from γG-receiving children or their subsets on PWM-induced proliferation and maturation of control B lymphocytes. Total T lymphocytes, present in E(+) populations isolated from children receiving γG therapy, were normally able to help allogeneic control B lymphocytes present in E(−) preparations to proliferate and to mature into ICC during a 7-d PWM-stimulated culture (Table IV). Such an effect was increased by a previous irradiation of E(+) leukocytes. The cell recovery and expression of sIg determinants were comparable to that of control cocultures. EAγ(+) leukocytes isolated either from treated children or from control individuals were unable to allow maturation of B lymphocytes ($P < 0.001$), unless they were irradiated in order to abolish the suppressor activity induced by fixation of EA complexes. EAγ(−) leukocytes from treated children, contrary to control EAγ(−) leukocytes, were ineffective as inducers of proliferation ($P < 0.02$) and maturation into plasma cells of control B lymphocytes ($P < 0.02$), even after a 2,000-rad irradiation.

Suppressor activity of T lymphocytes from γG-receiving children or their subsets on PWM-induced T and B cell proliferation and maturation of B lymphocytes. Addition of control E(+) leukocytes to a constant number of autologous or allogeneic total control leukocytes (ratio 1:1) did not affect proliferation and maturation of B lymphocytes in presence of PWM. In contrast, E(+) leukocytes from children under γG

TABLE III

PWM-induced Proliferation and Maturation into Plasma Cells of γG-treated Children E(−) Leukocytes Co-cultured with Control E(+) Leukocytes

Source of E(+) leukocytes	Source of E(−) leukocytes	No. of experiments performed	Number of recovered cells × 10^3±1 SD			
				Plasma cells containing		
			Total cells	IgM	IgG	IgA
Control	Control	25	159.4±50.3	10.1±5.1	8.1±4.6	7.6±3.9
Children receiving plasmatic γG	Treated children	7*	65.3±25.0	0.3±0.1	0.2±0.1	0.1±0.1
Control	Treated children	7*	51.0±32.7	0.1±0.1	0.1±0.1	0.1±0.1

PWM-stimulated co-cultures were performed in autologous or allogeneic situations. Co-cultures of E(+) and E(−) cells were performed using 160.10^3 E(+) leukocytes and 40.10^3 E(−) leukocytes. Isolated E(+) or E(−) leukocytes did not mature into plasma cells in presence of PWM.

* Identical results were obtained with E(−) leukocytes isolated from two children receiving placental γG.

therapy provoked a significant inhibition of T and B cell proliferation ($P < 0.001$) and of B cell maturation ($P < 0.001$) when added to control total leukocyte cultures (Table V). EAγ(+) leukocytes isolated from γG-treated children exerted a suppressor activity restricted to B cell maturation ($P < 0.001$) comparable to that observed with control EAγ(+) leukocytes, regardless of the ratio of EAγ(+) to target cells used (1:4 to 1:1). Whereas control EAγ(−) leukocytes added to allogeneic total leukocyte cultures did not affect proliferations and B cell maturation, the same cell populations isolated from γG-treated children exerted a suppressor effect on proliferations ($P < 0.01$) and strongly inhibited maturation of B lymphocytes ($P < 0.001$). This suppressor activity was also observed at lower ratios (1:4 and 1:2). A similar suppressive activity was obtained with NWC filtered E(+) or EAγ(−) populations (data not shown).

Properties of TS lymphocytes isolated from children receiving plasmatic γG. The suppressor activity of E(+) or EAγ(−) leukocytes from γG-treated children was totally abolished by a 2,000-rad irradiation

TABLE IV

Helper Function Exerted by E(+), EAγ(+), or EAγ(−) Lymphocytes from Control or Children Treated with Plasmatic γG Co-cultured with Control E(−) Population Stimulated by PWM

Source of E(+) leukocytes or subset co-cultured with control E(−) population	No. of experiments performed	No. of recovered cells × 10^3±1 SD			
			Plasma cells containing		
		Total cells	IgM	IgG	IgA
Control E(+)	25	159.4±50.3	10.1±5.1	8.1±4.6	7.6±3.9
Control irradiated E(+)	10	237.5±61.3	29.1±6.3	26.7±7.9	23.2±7.1
Treated children E(+)	7*	165.6±32.7	7.3±3.6	6.7±4.3	5.5±3.4
Treated children irradiated E(+)	6	195.0±34.1	22.4±8.9	21.0±7.7	19.2±5.6
Control EAγ(+)	7	118.7±15.0	0.4±0.4	0.2±0.1	0.3±0.3
Control irradiated EAγ(+)	5	112.0±19.7	9.6±1.2	9.3±3.9	7.2±2.1
Treated children EAγ(+)	5*	123.6±11.8	0.3±0.2	0.1±0.2	<0.1
Treated children irradiated EAγ(+)	2	116.5±23.3	6.4±0.1	5.3±0.6	5.4±0.4
Control EAγ(−)	10	144.0±41.5	14.1±4.2	13.9±4.7	14.3±4.5
Control irradiated EAγ(−)	4	171.5±17.2	25.2±5.6	18.4±3.1	16.7±4.4
Treated children EAγ(−)	5*	122.3±21.1	1.1±0.2	0.7±0.4	0.3±0.2
Treated children irradiated EAγ(−)	3	95.5±31.6	0.2±0.1	0.2±0.1	0.2±0.1

The helper function on B lymphocyte maturation was studied by co-culturing, in presence of PWM, 160 × 10^3 E(+), EAγ(+), or EAγ(−) leukocytes isolated from control or children receiving plasmatic γG and 40 × 10^3 allogeneic control E(−) cell populations. E(+) preparation or their subsets were either untreated or irradiated (2,000 rad).

* Helper effect of E(+) but not of non-irradiated EAγ(+) or EAγ(−) leukocytes isolated from two children receiving placental γG was also observed.

TABLE V
Suppressive Effect Exerted by E(+), EAγ(+), or EAγ(−) Lymphocytes from Control or Children Treated with Plasmatic γG Co-cultured with Control Total Leukocytes Stimulated by PWM

Source of E(+) leukocytes or subset co-cultured with control total leukocytes	No. of experiments performed	Number of recovered cells × 10^3±1 SD			
			Plasma cells containing		
		Total cells	IgM	IgG	IgA
Control E(+)	20	126.6±20.2*	7.6±4.6	6.6±1.9	6.8±3.4
Control irradiated E(+)	10	157.8±18.4	17.7±3.3	19.1±0.7	13.0±2.1
Treated children E(+)	5‡	76.6±15.2§	0.3±0.3	0.2±0.2	0.3±0.4
Treated children irradiated E(+)	5	167.2±14.8	12.9±2.3	9.3±2.6	9.0±3.9
Control EAγ(+)	15	152.5±38.9	0.5±1.0	0.4±0.3	0.2±0.1
Control irradiated EAγ(+)	4	114.5±10.7	7.1±0.3	8.2±2.3	5.9±2.1
Treated children EAγ(+)	5‡	125.5±28.2	0.6±0.5	0.2±0.2	0.3±0.1
Treated children irradiated EAγ(+)	3	118.7±41.6	6.6±1.4	5.7±0.7	4.3±2.0
Control EAγ(−)	15	136.3±24.0	6.1±2.6	5.5±1.7	5.6±1.4
Control irradiated EAγ(−)	4	137.7±29.9	12.4±4.7	10.8±3.6	8.8±4.9
Treated children EAγ(−)	5‡	81.5±16.2	0.6±0.5	0.3±0.4	0.2±0.1
Treated children irradiated EAγ(−)	3	114.5±14.7	5.1±1.1	4.8±0.9	4.7±0.5

The suppressor effect on B lymphocyte maturation was studied by co-culturing, in presence of PWM, 100×10^3 E(+), EAγ(+), or EAγ(−) leukocytes isolated from control or children receiving plasmatic γG and 100×10^3 allogeneic control total cell populations. E(+) preparations or their subsets were either untreated or irradiated (2,000 rad).
Percentage of recovered B lymphocytes detected by sIg studies and T lymphocytes revealed with an a-HTLA antiserum were, respectively, 14.7±6.8 and 40.5±8.7% in cultures with control E(+)* and 12.7±3.5 and 51.9±13.3% in cultures with treated children E(+).§
‡ The same suppressive effect was observed with E(+), EAγ(+), or EAγ(−) leukocytes isolated from two children receiving placental γG.

($P < 0.01$) (Table V) or by a preincubation (24 h 37°C) in culture medium added with normal human serum ($P < 0.001$) (data not shown). The suppressor activity exerted by EAγ(+) leukocytes from γG-treated children and from controls were similarly sensitive to irradiation ($P < 0.001$) but not to a previous incubation ($P > 0.5$). Although, as already indicated, E(+) leukocytes from γG-treated children exerted a helper activity on B lymphocytes present in control E(−) cell preparations, the same E(+) leukocytes became able to express a suppressive activity when a small quantity of control E(+) was added to the co-cultures. The intensity of this suppressive effect was proportional to the number of added control E(+) cells (Fig. 2). Cells present in control E(+) leukocyte preparations, and necessary for the expression of γG-treated E(+) leukocyte suppression, were isolated in EAγ(−) populations (Fig. 2) and resistant to a 2,000-rad irradiation (data not shown).

In vitro effect of γG-preparations on control leukocytes. Total control leukocytes, cultured in presence of either plasmatic or placental γG-preparation (0.15–1.5 mg/ml) used for therapy, were unable to proliferate ($P < 0.001$) and maturation of B lymphocytes was abolished ($P < 0.001$) (data not shown). In order to explain these results, separated cell populations were incubated in presence of γG before co-culturing with control E(−) or total leukocytes. The helper effect of incubated E(+) leukocytes could not be studied, however, since control E(+) leukocytes lost their helper function after a 48-h incubation in culture medium alone. As shown in Table VI, control fresh, but not preincubated or irradiated E(+) leukocytes acquired suppressor activity after 24 to 48 h (but not 1 h) incubation in presence of 0.07 to 1.5 mg/ml γG. This in vitro γG-induced suppressor activity was proportional to the dosage of γG. Cell cytotoxicity was observed over 1.5 mg/ml. Similar results were obtained when EAγ(−) leukocytes were incubated with γG. Because E(+) or EAγ(−) cell preparations contained few monocytes (≤5%), it was possible that monocytes were involved in the observed suppressive effect. In fact, an extensive monocyte depletion of E(+) or EAγ(−) leukocyte preparation on a NWC reduced the in vitro suppressive activity induced by plasmatic γG($P < 0.05$). Control E(+) or EAγ(−) leukocytes incubated in presence of plasmatic γG and an anti-PGE_2 antiserum, in order to inhibit PGE_2 that could be secreted by γG-activated cells, did not acquire any suppressive activity (Table VI). In contrast, the suppression exerted by γG-activated E(+) cells was insensitive to the addition of anti-PGE_2 antiserum in the test culture, suggesting that PGE_2 was only required in the activation phase of TS

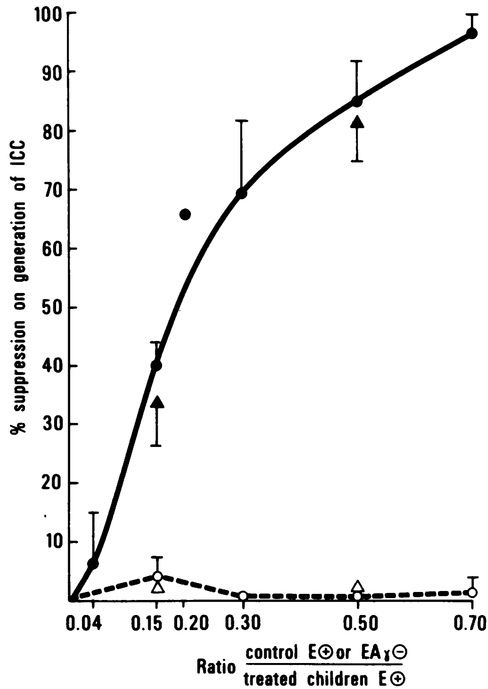

FIGURE 2 Suppressor activity of E(+) leukocytes from children receiving plasmatic γG (——) or from control (-----) on normal B lymphocyte maturation in the presence of increasing numbers of control allogeneic E(+) (mean of four experiments) or EAγ(−) leukocytes (mean of two experiments). PWM-stimulated co-cultures were performed with 40 × 10^3 control E(−) leukocytes and 160 × 10^3 γG-treated children E(+) leukocytes mixed with control E(+) (●) or EAγ(−) (▲) leukocytes added at varying ratios (0 to 0.75). Suppressor activity on generation of ICC was proportional to an increasing ratio of control E(+)-treated children's E(+) cells. No suppressor activity was obtained by mixing control E(+) leukocytes with allogeneic control E(+) (○) or EAγ(−) leukocytes (△).

(data not shown). On the other hand, control E(+) leukocytes did not acquire any suppressor activity when incubated with purified F(ab)'2 fragments of plasmatic γG or with plasmatic or placental γG preparations depleted of aggregates by gel filtration. Such deaggregated γG preparations treated by heat (56°C for 60 min, then 63°C for 20 min) in order to again provoke aggregates, became capable of inducing TS lymphocyte activation. Finally, plasmatic or placental γG preparations previously absorbed with total E(−) leukocytes and adjusted at 1 mg/ml retained their ability to induce TS activity (data not shown).

Control B lymphocytes present in E(−) preparations pretreated (1–4 h) by either plasmatic or placental γG(1 mg/ml) remained able to proliferate and to mature with the help of control, untreated T lymphocytes. However, when co-cultures were performed by mixing control irradiated E(+) leukocytes (in order to inhibit the γG-induced suppressive effect) and control E(−) leukocytes in presence of PWM and γG(1 mg/ml), proliferation ($P < 0.01$) and maturation of B lymphocytes ($P < 0.001$) were profoundly diminished. The suppressor effect of γG when present during the culture period was still observed when anti-PGE_2 substances (indomethacin or anti-PGE_2 antiserum) were added in order to prevent a possible indirect PGE_2-dependent suppression of B cell maturation (data not shown).

DISCUSSION

Repeated intramuscular injections of plasmatic or placental γG to 12 nonimmunodeficient children provoked a marked reduction of in vitro PWM-induced T and B lymphocyte proliferations and B lymphocyte maturation into plasma cells, an effect that persisted several months after cessation of therapy. This failure appears to be the consequence of an in vivo-induced dysregulation of both T and B cell functions.

Firstly, B lymphocytes present in E(−) leukocyte preparations responded poorly to a helper effect exerted by control or autologous T lymphocytes. Such an inability could be due either to an indirect effect, in relation to a suppressive activity exerted by monocytes, by the few T lymphocytes present in E(−) cell preparations, or to a direct inhibition of B lymphocytes. Indeed, it is well-known that activated monocytes are able to suppress lymphocyte proliferation (6, 11, 12) and B cell maturation (13, 14). However, depletion of monocytes from γG-treated children E(−) cell preparations or their replacement by normal monocytes did not improve B lymphocyte maturation. On the other hand, depletion of T lymphocytes of low affinity for sheep erythrocytes, which have been described as suppressor for the PWM-driven B lymphocyte maturation even in a normal situation (3, 27), did not restore the B lymphocyte function. These results suggest that a direct inhibition of B lymphocyte responses occurred in vivo, likely by fixation of γG on Fcγ receptors. However, after enzyme cleavage of γG (detected by immunofluorescence) from the membrane, B lymphocytes remained unable to respond, as if the strong in vivo inhibition does not allow, in our experimental conditions, an in vitro recovery. The interpretation of a direct inhibition of B lymphocytes is sustained by in vitro experiments showing that B lymphocytes were directly inhibited in the presence of γG throughout the culture. This inhibition was not complete however, because B lymphocytes could reach a certain degree of differentiation marked by the loss of membrane IgD, an event that was reported to be concomitant with B cell maturation (47), but were unable to express γ- and α-chain determinants.

Secondly, although T lymphocytes from γG-treated children were able to help the maturation of normal B lymphocytes (present in E[−] populations), they exerted a suppressor activity when added to unfractionated normal leukocytes. This suppressor activity

TABLE VI

Induction of T Lymphocyte Suppressor Activity by in vitro Incubation of Control Leukocytes in Presence of Plasmatic γG Preparation

Treatment of E(+) or EAγ(−) cell preparation added to total leukocyte cultures	No. experiments performed	No. of recovered cells × 10^3 ± 1 SD			
			Plasma cells containing		
		Total cells	IgM	IgG	IgA
E(+) incubated alone	25	121.3±33.0	8.9±4.9	7.8±1.2	8.0±2.7
E(+) incubated with γG	18*	75.2±26.3	0.8±1.6	0.7±1.4	1.0±1.7
E(+) irradiated, then incubated with γG	2	140.5±40.3	9.6±1.8	9.3±2.1	8.6±1.5
E(+) preincubated alone, then with γG	7	93.4±21.6	6.3±1.3	5.7±1.0	6.1±0.2
NWC filtrated E(+) incubated with γG	7	102.2±31.9	3.8±2.4	3.7±2.4	3.1±2.2
E(+) incubated with γG + aPGE_2	7	132.7±17.8	7.6±1.5	5.8±0.6	6.3±1.7
E(+) incubated with γG + aTXB_2	4	71.0±7.3	0.5±0.4	0.3±0.5	0.5±0.4
EAγ(−) incubated alone	7	152.5±16.9	9.3±1.4	7.8±1.9	7.7±1.7
EAγ(−) incubated with γG	7	84.7±26.0	0.7±0.6	0.4±0.3	0.6±0.5
EAγ(−) incubated with γG + aPGE_2	5	119.4±18.3	7.1±3.4	6.5±2.8	6.7±2.4
EAγ(−) incubated with γG + aTXB_2	2	65.7±10.6	0.6±0.2	0.8±0.3	0.4±0.3

The in vitro induction of suppressor activity by plasmatic γG preparation was tested on untreated, preincubated (24 h, 37°C), NWC filtered or irradiated (2,000 rad) control E(+) or EAγ(−) leukocytes (1 × 10^6/ml) incubated in presence of 1 mg/ml of γG for 48 h before being added to control allogeneic total leukocyte PWM-stimulated cultures. In some experiments, an anti-PGE_2 antiserum was present during the 48 h incubation with γG. A monospecific anti-thromboxane B_2 (aTXB_2) antiserum that did not crossreact with PGE_2, and which neutralized an important class of prostaglandin secreted by monocytes, was used as control in order to estimate a possible effect of immune complexes.

* Control E(+) leukocytes incubated in presence of placental γG (1 mg/ml) acquired the same suppressive activity.

was totally neutralized by irradiation, suggesting that suppression was displayed by in vivo activated TS lymphocytes that operated only when able to divide, as already mentioned for various TS lymphocyte activities (20, 21, 26, 48). In an attempt to further characterize in vivo γG-induced suppressor leukocytes, the respective activities of EAγ(+) and EAγ(−) populations were studied. EAγ(+) leukocytes from treated patients exerted a suppressor effect on B lymphocyte maturation but not on proliferation, an effect that appeared to be radiosensitive but resistant to a 24-h preincubation, as already described for normal individual EAγ(+) (5, 26). A suppressor effect found in EAγ(+) cells is not peculiar to γG-treated children since it is well known that technical procedures for isolation of EAγ(+) subset activated cells to become suppressive (5, 49). Titration experiments performed with varying numbers of EAγ(+) leukocytes did not allow detection of a greater suppressive effect exerted by the treated children subset. Thus, we are not able to discriminate between a possible in vivo activation of EAγ(+) by γG preparations and an artificial in vitro activation by EA complexes. EAγ(−) leukocytes from γG-treated children appeared to be more clearly activated in vivo because they exerted a spontaneous suppressive activity that was not detected in normal EAγ(−) leukocytes. The observation that suppressor activity was not altered by removal of NWC-adherent leukocytes suggests that T lymphocytes that do not bear Fcγ receptors were responsible for the effect. The in vivo γG-induced TS lymphocyte activity was comparable with that exerted by in vitro Con A-activated TS lymphocytes since they both affected proliferation and B cell maturation, and disappeared after incubation (9, 10, 26).

γG-induced TS activity appeared to be dependent on a cell-to-cell interaction. Indeed, the γG-induced suppression was only expressed in the presence of a normal, radioresistant, Fcγ(−) subset. It may be that leukocytes from treated children lack an intermediate cell activity necessary for expression of TS lymphocytes. Alternatively, as already proposed in other situations (17, 50), it may exist in treated children's leukocytes a "contra-suppressive" activity able to protect helper cells from the inhibitory effect of suppressor T cells, but which is overcome by addition of normal helper cells. Either hypothesis would explain why, despite activation of TS lymphocytes, a helper function was observed when treated children's E(+) cells were co-cultured with normal E(−) leukocytes since, in this condition, TS lymphocytes could not operate. When studied in Fcγ(+) or Fcγ(−) leukocyte subsets, the helper function normally found in Fcγ(−) populations was absent in treated children's leukocytes even after irradiation. In contrast, a helper function was observed in the irradi-

ated Fcγ(+) subset, both in treated children and in normal leukocytes. Fcγ(+) cell populations thus appear heterogeneous, since not only radiosensitive suppressor cells but also radioresistant T helper lymphocytes were found. Such heterogeneity, at least in terms of membrane phenotype, was recently mentioned by Reinherz et al. (51) and by Kaszubowski et al. (52).

The observation that γG preparation induced in vitro a TS activity comparable, in all respects, to that observed in γG-treated children helps, to some extent, to analyze the mechanisms involved. The activation, but not the expression, of TS lymphocytes appeared to be dependent on PGE_2 secretion by γG-activated cells. In fact, it has already been shown in mice (53) and suggested in humans (54) that PGE_2 can induce TS activity on T lymphocyte proliferation. We have reported elsewhere that PGE_2 can directly activate TS cells effective on human T and B cell proliferation and B lymphocyte maturation (55). Monocytes could be responsible for the in vitro PGE_2-induced TS activation, since activated monocytes were shown to produce large amounts of PGE_2 (56).

The exact mechanism of the in vivo and in vitro activation of TS lymphocytes is questionable. Anti-Ia antibodies that could be present in γG preparations might be responsible for the TS activation. Although anti-HLA, including anti-Ia antibodies were not detected in plasmatic γG preparations using a microcytotoxicity test, we performed absorption experiments in order to reduce the level of anti-Ia antibodies that might have not been detected by such an assay. Under our conditions of absorption, we failed to remove the induction of TS activity. We also showed that F(ab)'2 fragments of plasmatic γG failed to induce suppressive activity indicating that the effect was dependent on the Fc portion of γG. Finally, deaggregated γG preparation was inefficient in inducing TS activity unless it was aggregated again by heat. Altogether, these data tend to indicate that aggregated or denatured γG plays an important role in the dysregulation of the PWM responses, probably by its attachment on membrane Fcγ receptor, present on B lymphocytes, monocytes, and a T lymphocyte subset (5, 38, 56). B lymphocyte maturation could well be directly inhibited by fixation of IgG on membrane, as already described in mouse (57, 58). The same mechanism could activate monocytes to produce large amounts of PGE_2, as shown by Passwell et al. (56). Such an activation appears, however, transitory or not detected in blood monocytes since adherent cells from γG-treated children behave like control adherent cells. The activation of TS lymphocytes is likely to be secondary to the effect of PGE_2 secreted by activated monocytes, that acts on T lymphocytes and particularly on Fcγ(−) lymphocytes, as previously shown (55). Our observation that TS activity is predominantly found in Tγ(−) lymphocyte populations does not exclude, however, the possibility that Fcγ(+) TS lymphocytes could have been directly activated by γG, and thus lost their Fcγ receptors as already described (59). Finally, γG preparations inhibit helper function normally supported by Fcγ(−) cell subsets. Whether this lack of helper activity was secondary to the in vivo TS activity is not demonstrated. If so, one would expect that helper function found in EAγ(+) population was either insensitive to the in vivo TS functions or selected by our technical procedures.

The observation that aggregates seem to be responsible of the induced dysregulation does not exclude other possible mechanisms. Anti-Ia antibodies for instance, especially present in placental γG (60), might well induce comparable results as described in vitro by Broder et al. (61). Whatever the case, preparation of γG used for therapy should be depleted of aggregates as well as of anti-Ia antibodies. In the present study, we did not document any adverse effect of γG therapy despite the profound abnormalities described in the PWM system. The only in vivo modification was a return to normal of serum IgE levels in the three children who had an increased level before treatment. The absence of other abnormalities, including influence on the level of other serum immunoglobulin classes, could in part be explained by the fact that γG-induced TS activity was ineffective in absence of normal T lymphocytes. However, since B lymphocytes are affected by γG therapy, a longitudinal study of primary and secondary antibody formation to antigenic stimulations could reveal a possible in vivo effect on humoral function. Even if our study does not show any clear correlation between in vitro abnormalities and in vivo effects due to γG therapy, it indicates that an assay often used in the investigation of immune disorders can be profoundly modified during such a treatment. Although we have observed a spontaneously occurring TS lymphocyte activity in some common variable hypogammaglobulinemic patients before any γG therapy, it appears that caution should be taken in interpreting in vitro PWM dysfunction. Indeed, γG-induced immune dysregulation can mimic or amplify abnormalities due to primary physiopathological mechanisms.

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REFERENCES

1. Tadakuma, T., and C. W. Pierce. 1978. Site of action of a soluble immune response suppressor (SIRS) produced by

Concanavalin A activated spleen cells. *J. Immunol.* **117**: 967–972.
2. Bailleux, R. E., C. J. Heynen, F. Uytdehaag, and B. S. M. Zegers. 1979. Regulation of cell activity: role in T cells. *Immunol. Rev.* **45**: 3–39.
3. Fauci, A. S., and B. F. Haynes. 1979. Pokeweed mitogen-induced PFC responses of human peripheral blood lymphocytes: regulation of B cell triggering. *In* Antibody Production in Man. A. S. Fauci and R. Bailleux, editors. Academic Press, Inc., New York. 17–35.
4. Shou, L., S. A. Schwartz, and R. A. Good. 1976. Suppressor cell activity after Concanavalin A treatment of lymphocytes from normal donors. *J. Exp. Med.* **143**: 1100–1110.
5. Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydyard, and M. D. Cooper. 1977. Functional analysis of two human T-cell subpopulations: help and suppression of B-cell responses by T cells bearing receptors for IgM or IgG. *J. Exp. Med.* **146**: 184–200.
6. Laughter, A. H., and J. J. Twomey. 1977. Suppression of lymphoproliferation by high concentrations of normal human mononuclear leukocytes. *J. Immunol.* **119**: 173–182.
7. Sakane, T., and I. Green. 1977. Human suppressor T cells induced by Concanavalin A: suppressor T cells belong to distinctive T cell subclasses. *J. Immunol.* **119**: 1169–1178.
8. Fineman, S. M., F. B. Mudawwar, and R. S. Geha. 1979. Characteristics and mechanisms of action of the Concanavalin A-activated suppressor cell in man. *Cell. Immunol.* **45**: 120–132.
9. Hayward, A. R., L. Layward, P. M. Lydyard, L. Moretta, M. Dagg, and A. R. Lawton. 1978. Fc receptor heterogeneity of human suppressor T cells. *J. Immunol.* **121**: 1–5.
10. Haynes, B. F., and A. S. Fauci. 1978. Activation of human B lymphocytes. X. Heterogeneity of Concanavalin A-generated suppressor cells of the pokeweed mitogen-induced plaque forming cell response of human peripheral blood lymphocytes. *J. Immunol.* **121**: 559–565.
11. Bona, C., F. Audibert, D. Juy, and L. Chedid. 1976. Cell suppression in PPD-induced blast specific response of human peripheral blood lymphocytes. *Clin. Exp. Immunol.* **26**: 258–266.
12. Rice, L., A. H. Laughter, and J. J. Twomey. 1979. Three suppressor systems in human blood that modulate lymphoproliferation. *J. Immunol.* **122**: 991–996.
13. Goodwin, J. S., A. D. Bankhurst, and R. P. Messner. 1977. Suppression of human T cell mitogenesis by prostaglandin. Existence of a prostaglandin-producing suppressor cells. *J. Exp. Med.* **146**: 1719–1732.
14. Dimitriu, A., and A. S. Fauci. 1978. Activation of human B lymphocytes. IX. Modulation of antibody production by products of activated macrophages. *J. Immunol.* **120**: 1818–1824.
15. Knapp, W., and G. Baumgartner. 1978. Monocyte mediated suppression of human B lymphocyte differentiation in vitro. *J. Immunol.* **121**: 1177–1181.
16. Kurland, S. I., and R. Bockman. 1978. Prostaglandin E production by human blood monocytes and mouse peritoneal macrophages. *J. Exp. Med.* **147**: 952–961.
17. Reinherz, E. L., and S. F. Schlossman. 1979. Con A-inducible suppression of MLC: evidence for mediation by the TH_2^+T cell subset in man. *J. Immunol.* **122**: 1335–1341.
18. Stobo, J. D. 1977. Immunosuppression in man: suppression by macrophages can be mediated by interactions with regulatory T cells. *J. Immunol.* **119**: 918–925.
19. Bresnihan, B., and H. E. Jasin. 1977. Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosus. *J. Clin. Invest.* **59**: 106–116.
20. Siegal, F. P., and M. Siegal. 1977. Enhancement by irradiated T cells of human plasma cell production: dissection of helper and suppressor functions in vitro. *J. Immunol.* **118**: 642–647.
21. Gupta, S., and R. A. Good. 1977. Subpopulations of human T lymphocytes. II. Effect of thymopoietin, corticosteroids and irradiation. *Cell. Immunol.* **34**: 10–18.
22. Pelus, L. M. 1977. Prostaglandins and the immune response. *Life Sci.* **20**: 903–912.
23. Gelfand, E. W., H. M. Dosch, D. Hastings, and A. Shore. 1979. Lithium: a modulator of cyclic AMP-dependent events in lymphocytes. *Science (Wash. D. C.).* **203**: 365–367.
24. Olding, L. B., K. Benirschke, and M. B. A. Oldstone. 1974. Inhibition of mitosis of lymphocytes from human adults by lymphocytes from human newborns. *Clin. Immunol. Immunopathol.* **3**: 79–89.
25. Hayward, A. R., and P. M. Lydyard. 1978. Suppression of B lymphocyte differentiation by newborn T lymphocytes with an Fc receptor for IgM. *Clin. Exp. Immunol.* **34**: 374–378.
26. Durandy, A., A. Fischer, and C. Griscelli. 1979. Active suppression of B lymphocyte maturation by two different newborn T lymphocyte subsets. *J. Immunol.* **123**: 2644–2650.
27. Haynes, B. F., and A. S. Fauci. 1979. Activation of human B lymphocytes. XIII. Characterization of multiple populations of naturally occurring immunoregulatory cells of polyclonally induced in vitro human B cell function. *J. Immunol.* **123**: 1284–1298.
28. Kinkelstein, M. S., and J. W. Uhr. 1964. Specific inhibition of antibody function by passively administered 19S and 7S antibody. *Science (Wash. D. C.).* **146**: 67–70.
29. Waldmann, T. A., R. M. Blaese, S. Broder, and R. S. Krakauer. 1978. Disorders of suppressor immunoregulatory cells in the pathogenesis of immunodeficiency and auto-immunity. *Ann. Intern. Med.* **88**: 226–238.
30. Siegal, F. R., M. Siegal, and R. A. Good. 1976. Suppression of B cell differentiation by leukocytes from hypogammaglobulinemic patients. *J. Clin. Invest.* **58**: 109–122.
31. Griscelli, C., A. Durandy, D. Guy-Grand, F. Daguillard, C. Herzog, M. Prunieras. 1978. A syndrome associating partial albinism and immunodeficiency. *Am. J. Med.* **65**: 691–701.
32. Schultze, H. E., and J. F. Heremans. 1966. Nature and Metabolism of Extra-cellular Proteins. Elsevier-Dutton Publishing Co., Inc., New York. 236–317.
33. Colombani, J., and M. Colombani. 1974. Serologic recognition of histo-compatibility antigens using complement fixation. *Semin. Hematol.* **11**: 273–280.
34. Rivat, L., C. Rivat, M. Daveau, and C. Ropartz. 1977. Comparative frequencies of anti IgA antibodies among patients with anaphylactic transfusion reactions and among normal blood donors. *Clin. Immunol. Immunopathol.* **7**: 340–348.
35. Digeon, M., M. Laver, J. Riza, and J. F. Bach. 1977. Detection of circulating immune complexes in human serum by simplified assays with polyethylene glycol. *J. Immunol. Methods.* **16**: 165–183.
36. Jondal, M., G. Holm, and H. Wigzell. 1972. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. Exp. Med.* **136**: 207–215.
37. Grey, H. M., E. Rabellino, and B. Pirofsky. 1971. Immunoglobulins on the surface of lymphocytes. IV. Distribution

in hypogammaglobulinemia, cellular immune deficiency, and chronic lymphatic leukemia. *J. Clin. Invest.* **50:** 2368–2375.

38. Dickler, H. B., and H. G. Kunkel. 1972. Interaction of aggregated γ-globulin with B lymphocytes. *J. Exp. Med.* **136:** 191–196.
39. Janossy, G., A. H. Goldstone, D. Capellaro, M. F. Greaves, J. Kulenkampff, M. Pippard, and K. Welsh. 1977. Differentiation-linked expression of P28, 33 (Ia like) structures on human leukaemic cells. *Br. J. Haematol.* **37:** 391–402.
40. Preudhomme, J. L., and G. Flandrin. 1974. Identification by peroxydase staining of monocytes in surface immunofluorescence. *J. Immunol.* **113:** 1650–1653.
41. Horwitz, D. A., A. C. Allison, P. Ward, and N. Knight. 1977. Identification of human mononuclear leukocyte populations by esterase staining. *Clin. Exp. Immunol.* **30:** 289–298.
42. Dray, F., B. Charbonnel, and J. Maclouf. 1976. Primary prostaglandins in human peripheral plasma by radioimmunoassay. *Adv. Prostaglandin Thromboxane Res.* **1:** 93–97.
43. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3:** 645–649.
44. Natvig, J. B., and M. W. Turner. 1971. Localisation of Gm markers to different molecular regions of the Fc fragment. *Clin. Exp. Immunol.* **8:** 685–700.
45. Banker, C. A., and C. W. Cotman. 1972. Measurement of free electrophoretic mobility and retardation coefficient of protein-sodium dodecyl sulfate complexes by gel electrophoresis. *J. Biol. Chem.* **247:** 5856–5870.
46. Ferreira, S. H., S. Moncada, and J. R. Vane. 1971. Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nat. New Biol.* **231:** 237–240.
47. Preudhomme, J. L. 1977. Loss of surface IgD by human B lymphocytes during polyclonal activation. *Eur. J. Immunol.* **7:** 191–195.
48. Haynes, B. F., and A. S. Fauci. 1978. Activation of human lymphocytes. V. Kinetics and mechanisms of suppression of plaque forming cell responses by Concanavalin A-generated suppressor cells. *J. Immunol.* **120:** 700–708.
49. Le Thi Bich Thuy, and J. Brochier. 1979. Human B cell differentiation. II. Suppression by T cells of T-dependent and T independent plasma cell maturation. *J. Immunol.* **122:** 1842–1848.
50. Green, D. R., D. B. Murphy, H. Cantor, and R. V. Gersmon. 1980. Contrasuppression: generation and characterization of the effector. *IV Int. Congr. Immunol. (Paris)* (Abstr.).
51. Reinherz, E. L., L. Moretta, M. Ropers, J. M. Breard, M. C. Mingari, M. D. Cooper, and S. Schlossman, 1980. Human T lymphocyte subpopulations defined by Fc receptors and monoclonal antibodies. A comparison. *J. Exp. Med.* **151:** 969–971.
52. Kaszubowski, P. A., J. S. Goodwin, and R. C. Williams. 1980. Ia antigen on the surface of a subfraction of T cells that bear Fc receptors for IgG. *J. Immunol.* **124:** 1075–1078.
53. Webb, D. R., and I. Nowowiejski. 1978. Mitogen-induced changes in lymphocyte prostaglandin levels: a signal for the induction of suppressor cell activity. *Cell. Immunol.* **41:** 72–85.
54. Goodwin, J. S., R. P. Messner, and G. T. Peake. 1978. Prostaglandin suppression of mitogen-stimulated lymphocytes in vitro. Changes with mitogen dose and preincubation. *J. Clin. Invest.* **62:** 753–760.
55. Fischer, A., A. Durandy, and C. Griscelli. Role of prostaglandin E_2 in the induction of non-specific T lymphocyte suppressive activity. *J. Immunol.* In press.
56. Passwell, J., J. M. Dayer, and E. Merler. 1979. Increased prostaglandin production by human monocytes after membrane receptor activation. *J. Immunol.* **123:** 115–120.
57. Ryan, J. L., and P. A. Henkart. 1976. Fc receptor-mediated inhibition of murine B lymphocyte activation. *J. Exp. Med.* **144:** 768–775.
58. Hoffman, M. K. 1980. Antibody regulates the cooperation of B cells with helper cells. *Immunol. Rev.* **49:** 79–80.
59. Moretta, L., M. C. Mingari, and C. A. Romanzi. 1978. Loss of Fc receptor for IgG from human T lymphocytes exposed to IgG immune complexes. *Nature (Lond.).* **272:** 618–620.
60. Brochier, J., M. Bonneau, M. Robert, C. Samarut, J. P. Revillard, and J. Traeger. 1979. Anti-HLA DR allo-antibodies eluted from human placental tissue. *Transplant. Proc.* **11:** 779–782.
61. Broder, S., D. L. Mann, and T. A. Waldmann. 1980. Participation of suppressor T cells in the immuno-suppressive activity of a hetero antiserum to human Ia-like antigens (p28, 30). *J. Exp. Med.* **151:** 257–262.