Studies of T- and B-Cell Interactions in Adult Patients with Combined Immunodeficiency

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ABSTRACT Cellular interactions involved in the pathogenesis of hypogammaglobulinemia were studied in six patients with common variable immunodeficiency. Amounts of immunoglobulin (Ig)G and IgM in the supernate of pokeweed mitogen-stimulated cocultures of normal and immunodeficient mononuclear cells were measured by radioimmunoassays. Mononuclear cells from three of six patients inhibited Ig production of normal B cells ($P < 0.005$). When purified patient and normal T cells were added to B cells in various autologous or allogeneic combinations, it was observed that immunodeficient T cells (AT) from four patients suppressed normal IgM synthesis. Allogeneic normal T cells did not provide help for B cells from these same immunodeficient patients. In two patients, autologous T cells were able to help autologous B-cell IgM synthesis in vitro. In five patients, AT cells inhibited normal B-cell IgG synthesis. Removal of T cells bearing Ia determinants or T cells with Fc-IgG receptors did not diminish the suppressive effect of AT cells on normal B-cell Ig synthesis. Addition of indomethacin, a prostaglandin synthetase inhibitor, did not abrogate the suppressive effect of immunodeficient mononuclear cells. Addition of hydrocortisone succinate (10 μM) did reverse the suppressive effect of AT cells on IgM production in one patient; however, no in vitro reversal of suppressor cell effect was recorded in five. Suppression by immune-deficient T cells was eliminated by 2,000 rad of x-ray irradiation in three patients. After x-ray irradiation immune-deficient T cells could function as helpers of normal B cells.

INTRODUCTION

Patients with various forms of immune deficiency present fascinating experiments of nature which may eventually provide definitive insight into important features of immune regulation. The potential role of suppressor thymus-derived (T) lymphocytes in pathogenesis of common variable hypogammaglobulinemia was first presented by Waldmann et al. (1) on the basis of studies using co-cultures of patients' lymphocytes or isolated T cells and peripheral blood lymphocytes from normals in the presence of pokeweed mitogen. Some patients with common variable hypogammaglobulinemia may show normal proportions of peripheral blood bone marrow-derived (B) lymphocytes bearing surface immunoglobulin (Ig)[1] but are incapable of manufacturing circulating endogenous antibody. On the other hand, some patients with congenital agammaglobulinemia are apparently capable of generating a low but definite number of specific plaque-forming cells in vitro despite the absence of B lymphocytes in peripheral blood (2). These latter observations suggested that individuals with congenital agammaglobulinemia possessed a small number of pre-B cells capable of differentiation into Ig-producing cells. A number of studies indicate that the broad spectrum of immune deficiencies recorded in various patients represents a variety of intrinsic defects either in immune system differentiation or in abnormalities of cell to cell interaction (3–6).

Recent studies on the functional T-cell subsets Tγ and Tμ bearing receptors for the Fc portion of IgG and IgM, respectively (7–9), have indicated that Tγ cells may function as suppressor cells and Tμ as helper cells under certain experimental conditions. However, analyses of relative proportions and numbers of Tγ or Tμ cells among heterogeneous groups of immune-deficient patients have not clearly answered the role of these T lymphocytes in the pathogenesis of these immunodeficiency states (10–12).

The Ia antigen has been identified as an important

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Received for publication 17 May 1979 and in revised form 9 October 1979.

[1] *Abbreviations used in this paper*: Ig, immunoglobulin; MNC, peripheral blood mononuclear cells; PGE, prostaglandins of the E series.

differentiation structure in human B cells as well as in a number of other cells of hematopoietic origin (13–17). Lymphocytes bearing Ia antigens are absent in patients with infantile agammaglobulinemia (18); however, recently some evidence has been presented that T cells bearing Ia antigens may be involved in various experimental systems in suppressor (19–21) or helper activity (22). During the course of study of cell surface markers in a large group of immune-deficient patients, we noted that a certain proportion of such individuals showed markedly increased proportions of Ia-positive T cells.[2] Part of the present study was directed at the possible functional implications of such a change. However, the major objective of the current work was to assay T- and B-cell interactions among a group of adult patients with common variable immunodeficiency using sensitive in vitro Ig synthesis assays, and to dissect some of the immunological defects in such patients.

METHODS

Patients studied. A group of six adult patients with common variable immune deficiency formed the major clinical basis for the present work. A profile of these patients is shown in Table I. All but patient 6 were receiving parenteral gamma globulin once a month, or in some instances on a biweekly basis. Of interest was the fact that proportions of Ia(+) T cells varied markedly in many immunodeficient patients. This was found by using the same reagents and cell surface marker analysis throughout and did not appear to be correlated in any discernable way with their clinical status. No similar variation was recorded with normal control subjects studied concurrently.

Lymphocyte cell surface markers. Blood was collected by venipuncture in preservative-free heparin (1:1,000). Peripheral blood mononuclear cells (MNC) were separated on Ficoll-Hypaque gradients (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) (23) and washed three times with Hanks' balanced salt solution. T lymphocytes were detected by E-rosette formation using neuraminidase-treated erythrocytes (24). B lymphocytes were determined by a combination of surface marker assays including EAC-rosette formation (25), EA, rosette formation (26), and fluorescein-labeled rabbit $F(ab')_2$ anti-human $F(ab')_2$ (26, 27). Lymphocyte subpopulations enriched for T or B cells were obtained using neuraminidase-treated sheep erythrocytes and centrifugation of rosetting cells through a Ficoll-Hypaque gradient. Nonrosetting cells left at the Ficoll-Hypaque interface were considered to represent B cells plus null cells. Cells forming E rosettes consisted of lymphocytes that produced 90–95% E rosettes on reassay after separation. Cells that did not form E rosettes contained no >5% E rosettes on reassay. Cell viability was >95% in all preparations as determined by trypan blue dye exclusion.

[2] Williams, R. C., Jr., A. D. B. Webster, and M. F. Greaves. Manuscript in preparation.

TABLE I

Clinical Profile of Six Patients with Common Variable Hypogammaglobulinemia Studied

Patient	Sex and age	Serum IG levels*			Percent Serum Ig(+) peripheral blood lymphocytes	T cells	Percent		Ia(+) T cells§	Miscellaneous	Skin test reactivity‖
		IgG	IgA	IgM			Tγ‡	Tμ‡			
		mg/100 ml							%		
1	♀ 51	220	48	40	6	89	16	39	17–50	Bronchiectasis severe repeated bronchopulmonary infection	All negative
2	♂ 68	115	0	0	4	73	11	53	5–25	Chronic bronchitis	All negative
3	♂ 26	108	24	15	1	77	3	16	6–32	Urethral-rectal fistula with chronic perineal suppuration 1.5 yr	All negative
4	♂ 67	0	0	0	1	79	24	19	3–6	Chronic sinusitis and bronchitis	All negative
5	♂ 42	140	0	16	3	67	3	45	4–35	Bronchitis; repeated bronchopulmonary infections concomitant pernicious anemia	Mumps 8/24 h 3/48 h
6	♀ 52	315	21	17	6	82	16	48	4–46	Sudden onset of *Listeria* meningitis	All negative

* Normal control values for immunoglobulins: IgG 1,278±36 mg/100 ml; IgA 282±128 mg/100 ml; and IgM 109±49 mg/100 ml.

‡ Tγ cells normal mean 9.1±4.7%; Tμ cells normal mean 48±9.0%.

§ Normal mean Ia(+) T cells 2.4±1.2%. In some patients studied proportions of Ia(+) T cells varied considerably over a 6-mo period.

‖ All patients were tested with purified protein derivative, mumps, coccidioidin, streptokinase-streptodornase, candida and trichophytin. Skin test results expressed as millimeters of skin reactivity at 24 and 48 h.

The proportions of Tγ and Tμ cells in immunodeficient patients were determined as previously described (7–9, 27). In addition, T cells showing presence of human Ia antigens were determined after E rosetting of lymphocytes, incubation of isolated T cells for 12 h in 5% CO_2-air at 37°C, and staining with $F(ab')_2$ fragments of rabbit anti-human Ia antiserum. The rabbit anti-human Ia antiserum was prepared using a well-characterized rabbit anti-human Ia reagent generously provided by Dr. R. J. Winchester, The Rockefeller University, New York. Immune precipitates between known rabbit anti-Ia antiserum and lysates of Ia-positive cultured B-cell line membranes emulsified with complete Freund's adjuvant were injected at 2-wk intervals for a total of four to six injections into rabbits. Antisera were repeatedly absorbed with fresh suspensions of living human thymocytes obtained from children undergoing cardiac surgery until no background staining of human thymocytes or cultured T-cell lines was observed. $F(ab')_2$ pepsin fragments were prepared from absorbed rabbit anti-human Ia antisera using two successive 12-h pepsin digestions of whole antiserum with 2 mg of pepsin/ml of antiserum and final clearing by ultracentrifugation before use in cell surface marker analysis. Parallel studies with 50 normal adult controls showed that the mean for Ia positive (+) T cells was 2.4±1.2% in this population. Normal means for Tγ and T cells determined in the same group are shown in Table I.

Cell culture and co-culture techniques. MNC were initially adjusted to 1.0×10^6/ml in RPMI 1640 medium with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.) and 0.1 mg/ml of gentamicin. Cell suspensions were incubated for 7 d in the presence of an optimal concentration of pokeweed mitogen (1:200 dilution, Grand Island Biological Co.). Several lots of fetal calf serum were tested for cytotoxicity and the optimal one used in all subsequent experiments. Cells were cultured in microculture plates (250 μl/well, Linbro Chemical Co., Hamden, Conn.) and maintained at 37°C in 5% CO_2-air mixture for 7 d. In mixture experiments between patients and normal MNC, equal numbers of cells were mixed (final cell concentration, 3.0×10^5 in 250 μl). In other instances 1 μM indomethacin or 10 μM hydrocortisone succinate (both from Sigma Chemical Co., St. Louis, Mo.) was added to the culture medium. Controls for addition of indomethacin included cells incubated in RPMI-fetal calf serum with 0.01% ethanol as previously described (28). In these instances presence of this concentration of ethanol did not affect control or experimental results (28). When lymphocyte fractions enriched for T or B cells were studied, B-lymphocyte fractions were adjusted to 6×10^4 cells/microplate well and to this constant number of B cells, varying numbers of T cells were added. The total volume in each microwell was maintained at 250 μl. After 7 d, plates were centrifuged (500 *g* for 10 min) and all supernate collected for radioimmunoassay of supernatant Ig produced.

During the course of many experiments an attempt was made to determine the effect of various T-cell subsets on Ig synthesis. In this regard Tγ cells were prepared as originally described by Moretta et al. (8, 9). These Tγ putative suppressor cells were removed by EA rosetting from previously T cell-enriched preparations. In addition, T-cell fractions were treated with highly specific undigested rabbit anti-human Ia antiserum in the presence of fresh rabbit complement to eliminate the small fraction in normals or the larger fraction in combined immunodeficiency patients of Ia(+) T cells. Controls for such treatment included normal rabbit serum plus complement alone.

Radioimmunoassay for supernatant IgM and IgG. Flat bottom flexible microtiter plates (Cooke Engineering Co., Alexandria, Va.) were used for solid-phase radioimmunoassays. 100 μl of purified IgG or IgM (20 μg/ml in phosphate-buffered saline containing 0.01% sodium azide) was placed in the wells. Plates were incubated 1 h at 37°C and 16 h at 4°C. After incubation, wells were washed three times with phosphate-buffered saline and dipped in gelatin buffer (phosphate-buffered saline, 0.03% gelatin, 0.01% sodium azide), and after 2 h at room temperature, plates were washed once with Tween buffer (Atlas Chemical Industries, Inc., Wilmington, Del.). The Tween buffer consisted of 0.05% vol/vol Tween in phosphate-buffered saline containing 0.01% sodium azide. 50 μl of supernate was placed in the wells and 50 μl of ^{125}I-labeled anti-IgG or IgM was added. Plates were incubated 30 min at 37°C and 30 min at 4°C for IgG, or 12 h at 4°C for IgM. Wells were then washed three times with Tween buffer and the individual well cut out with scissors and counted in a gamma counter. Results were compared with a standard curve prepared by doubling dilutions of purified Ig (10 μg/ml–10 ng/ml). Exact amounts of Ig in supernates were then calculated by computer. The computer program had two parts. First, a polynomial regression of the standard curve was established from standard dilutions, and second, each count was calculated by the polynomial regression.

Human IgG was purified from human gamma globulin fraction II (Pentex Biochemical, Kankakee, Ill.) by DEAE chromatography. Human IgM was isolated from six pooled Waldenstrom patients' plasmas using a euglobulin precipitation method and final DEAE chromatography. Purity of the antisera was verified by immunodiffusion using isolated Ig components. Rabbit anti-IgG and anti-IgM were isolated by passage of monospecific serum through appropriate immunoabsorption columns and elution of specific antibody before labeling. The purified monospecific anti-Ig antibodies were radiolabeled with ^{125}I by the chloramine T method (29).

Irradiation of cells. In some experiments immunedeficient T cells were irradiated (2×10^6 cells/ml in RPMI 1640 with 10% fetal calf serum) with 1,000 or 2,000 rad (223 rad/min).

RESULTS

Effect on Ig synthesis by normal MNC after addition of MNC from patients with combined immune deficiency. Preliminary screening experiments used allogeneic co-cultures of 16 normal, unrelated adult subjects with radioimmunoassay of IgG and IgM in supernates after 7 d of co-culture. These experiments showed no significant alteration in expected IgG or IgM synthesis from that predicted. In particular, such allogeneic combinations resulted in no significant mean decrease in IgG or IgM produced.

When MNC from six different patients with combined immune deficiency were co-cultured with MNC from normal subjects, Ig synthesis of normal B cells interacting in the test system was often suppressed. In Fig. 1, it can be seen that patients 1, 2, and 3 showed significant suppression of IgG and IgM synthesis in four different experiments ($P < 0.005$). The predicted amount (P) indicated by vertical brackets in the figures was calculated using the following formula: Normal Ig production + Patient Ig production ÷ 2 = P. On the other hand, in patient 5, IgG synthesis was

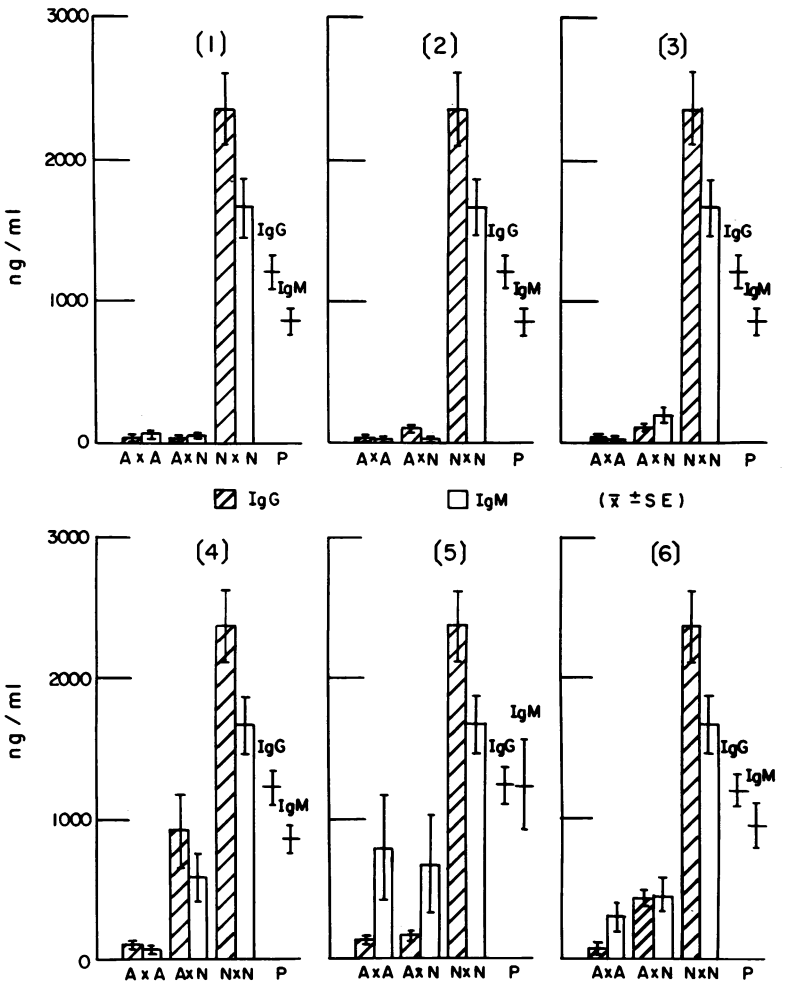

FIGURE 1 Ig production by mixtures of normal and combined immune-deficient patient MNC. A × A, culture of patient cells only; N × N, culture of normal cells only; A × N, culture of equal numbers of normal and patient cells. P = predicted Ig production of A × N indicated by brackets. All results are expressed as mean±SE Ig produced by 3×10^5 MNC (four different normals) in nanograms per milliliter. Each bar represents the results of at least four triplicate experiments.

suppressed but IgM was unaffected. Patients 4 and 6 did not show significant suppression of either normal IgG or IgM in co-culture experiments ($P > 0.05$). Even though patient 4 overall did not show suppression of three normal donors studied at different times over an 11-mo interval, considerable variation was recorded in the ability of lymphocytes from patient 4 to suppress lymphocyte Ig synthesis by different donors. Of interest was what appeared to be an apparent cyclical variation of activity by MNC from patient 4 in suppression of Ig synthesis by co-cultured normal lymphocytes. Some suggestion was provided by cumulative serial data that intrinsic synthesis of autologous Ig by lymphocytes from patient 4 was inversely correlated with the degree of suppression of this patient's MNC for normal lymphocyte Ig synthesis in co-culture.

Effect of addition of purified or enriched T- or B-MNC populations on Ig production. To investigate the existence of a putative suppressor cell in patients with combined immunodeficiency, T cells from immune-deficient patients were added to normal adult B cells in co-cultures. T cells from patients 1–5 suppressed IgM synthesis in mixed cultures with four different normal allogeneic B cells ($P < 0.05$) (Fig. 2). This was noted using a wide range of ratios of patient T cells to normal B cells. In parallel experiments, addition of normal T cell-enriched populations to normal B cells resulted in help and an increase in IgM synthesis in a dose-dependent fashion. Further-

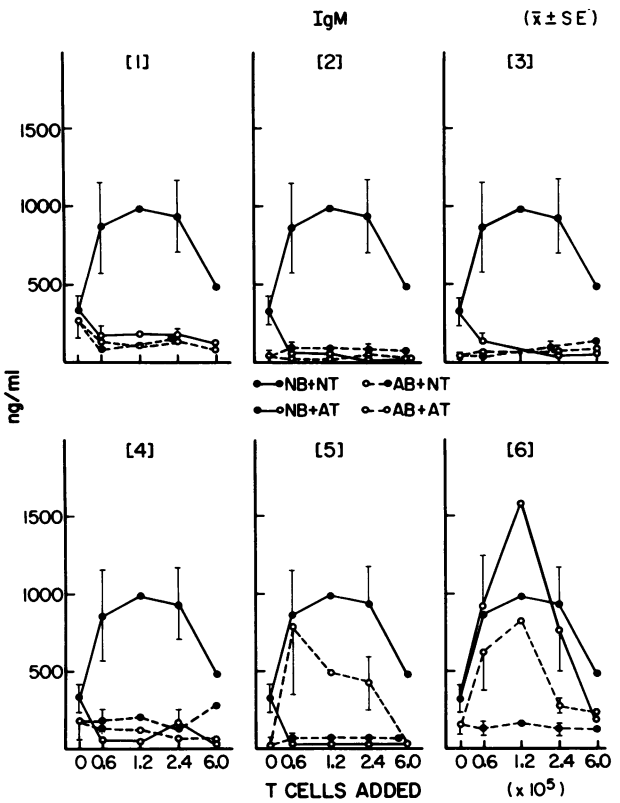

FIGURE 2 The effect on IgM production resulting from the addition of T cells to enriched B-cell suspensions. NB, normal B cells; NT, normal T cells; AB, immune-deficient B cells; and AT, immune-deficient T cells. Results are expressed as mean±SE, IgM produced by 0.6×10^5 B cells in nanograms per milliliter of co-cultures of patients with four different normal subjects. AT from patients 1–5 significantly inhibited NB IgM production ($P < 0.05$). To avoid cluttering, the bars indicating SE have been omitted around some points but in such instances the SE was of the same magnitude as the other points.

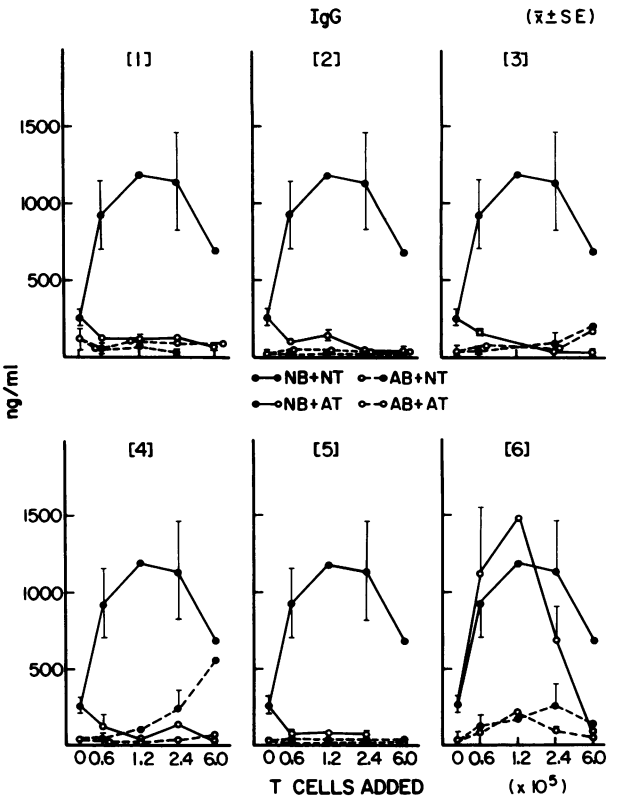

FIGURE 3 The effect on IgG production resulting from the addition of T cells to enriched B-cell suspensions. NB, normal B cells; NT, normal T cells; AB, immune-deficient B cells; and AT, immune-deficient T cells. Results are expressed as mean±SE, IgG produced by 0.6×10^5 B cells in nanograms per milliliter in co-cultures of patients with four different normal subjects. AT from patients 1–5 significantly inhibited NB IgG production ($P < 0.025$). To avoid cluttering, the bars indicating SE have been omitted around some points but in such instances the SE was of the same magnitude as the other points.

more, the addition of enriched normal T cells to these six immunodeficient patients' B-cell fractions did not result in significant help or any augmentation of IgM production (Fig. 2). In the case of patients 5 and 6, addition of immunodeficient subjects' separated T cells to autologous B cells, however, did lead to augmentation of in vitro IgM synthesis. When T cells from patient 5 were added to normal allogeneic B cells, no helper effect was observed. By contrast, when T cells from patient 6 were added either to allogeneic normal B cells or autologous B cells, IgM production was significantly enhanced ($P < 0.025$).

In Fig. 3 similar experiments involving co-culture of syngeneic- and allogeneic-enriched normal or immunodeficient patients' T- or B-lymphocyte fractions are shown with respect to IgG synthesis. Five patients (1–5) behaved similarly in that addition of their T cell-enriched fractions to normal B cells showed suppression of IgG synthesis in four different experiments ($P < 0.025$). However, in patient 6 purified or enriched T cells caused augmentation of IgG production by co-cultured normal B cells. In all immunodeficient patients studied, addition of allogeneic normal T cells did not enhance or help IgG production by B cells of immunodeficient patients. The decrease in Ig production of normal control seen with higher T cell ratios was a result of cell crowding.

Effect of rabbit anti-Ia antiserum on T cells. To investigate the possibility that T cells with surface Ia antigens might function as significant suppressor or helper cells, various combinations of normal or immunodeficient patients' B and T cells were studied in co-culture before and after lysis of T cells bearing Ia antigens. Results of these experiments are summarized in Fig. 4. In three instances when normal T and B cells were co-cultured before and after com-

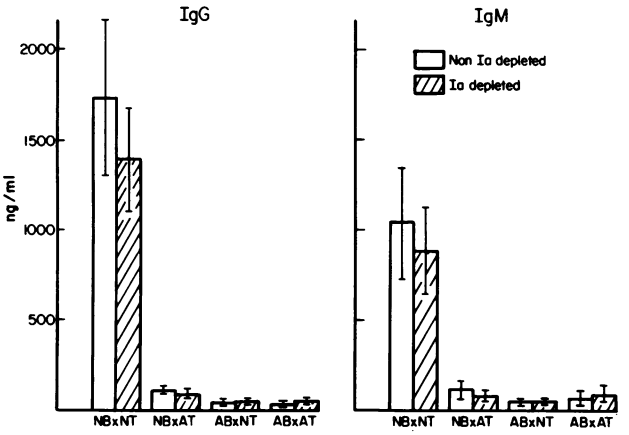

FIGURE 4 The effect of the removal of cells with surface Ia determinants on Ig production. NB, normal B cells; NT, normal T cells; AB, patient B cells; AT, patient T cells. Results are expressed as mean±SE, Ig produced by 0.6 × 10^5 B cells in nanograms per milliliter in co-cultures of a patient with five different normals (equal numbers of patients and normal cells).

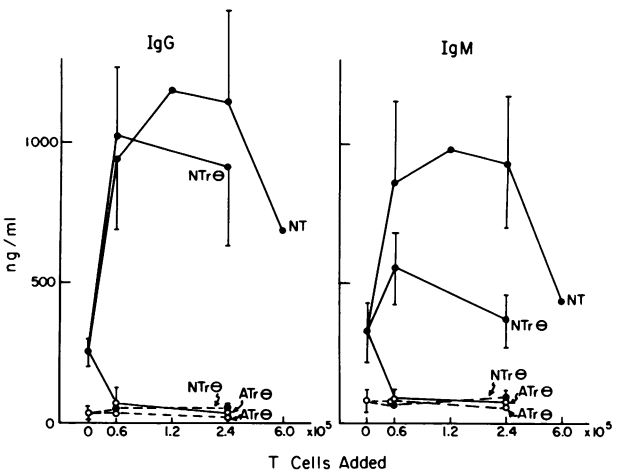

FIGURE 5 The effect of the depletion of Tγ cells on Ig production. Various numbers of T cells were added to a fixed number of B cells (0.6 × 10^5). NT, normal T cells; AT, patient T cells. Tγ θ indicates that T cells with Fc IgG receptors were removed. Results are expressed as mean ±SE, nanograms per milliliter of Ig produced by 0.6 × 10^5 B cells in co-cultures of six immunodeficient patients with normal donors. Removal of Tγ cells from NT suspensions resulted in a significant decrease in IgM production ($P < 0.05$). The patients' T cells did not change the suppression even after the depletion of Tγ cells.

plement-mediated lysis of Ia-bearing T cells, slight diminution of IgG synthesis was recorded ($P < 0.025$), but in two experiments no significant change was noted. The cytotoxic effect of the anti-Ia antiserum was shown by the fact that 36.5±4.7% of immune-deficient and 16.0±4.6% of normal T cells were killed by complement-mediated lysis. Rabbit complement alone was a control and showed only minor cytotoxicity (<10%). The results shown in Fig. 4 represent the mean value of these five experiments and show no overall significant decrease in Ig production. Furthermore, in allogeneic combinations of normal B cells and immunodeficient patients' T cells, patients' B cells and normal T cells, or patients' B cells and patients' T cells, no significant change in total Ig production was noted after lysis of Ia(+) T cells by anti-Ia antiserum and complement before introduction of T cells into culture.

Elimination of Tγ cells. Because Tγ cells have been implicated as suppressor cells in certain human systems, an attempt was next made to study cellular interactions in the basic lymphocyte co-culture system using T cell-enriched populations before and after elimination of Tγ cells by EA rosetting. The results of these six experiments are shown in Fig. 5. It can be seen that when purified normal T cells were added to normal B-cell fractions, a helper effect on both IgG and IgM synthesis was seen. When normal T cells (depleted of Tγ cells) were added to normal B cells, no significant change in IgG synthesis was apparent in comparison with experiments performed with non-depleted T-cell populations. In parallel experiments when IgM synthesis was examined, removal of Tγ cells from normal T-cell suspensions actually resulted in some relative diminution of IgM production ($P < 0.05$). In all cases even when immune-deficient T cells were depleted of Tγ cells, these depleted T cells would not help normal or autologous B cells and a suppressive effect on normal B cells was still observed. These data provided no evidence for an active suppressor role for Tγ cells using these in vitro assays.

Effect of indomethacin and corticosteroids. Because prostaglandins of the E series (PGE) have been shown to modulate immune function in other assay systems (28), we decided to examine the possibility that PGE synthesis might be involved in active suppression by T cells from some patients with combined immune deficiency. In all patients studied, addition of indomethacin (μM) did not reverse the inhibition of normal IgG or IgM production seen in co-cultures of normal and immunodeficient patients' MNC.

Moreover, when hydrocortisone succinate (10 μM) was added to identical co-culture assays, no reversal of IgG production was recorded in any of the six patients against four normal donors (Fig. 6). In contrast to this result in one of these patients, 5, a marked increment in IgM production was noted after hydrocortisone addition in two combinations of normal and patient MNC. However, the other immunodeficient patients' suppression of normal IgM production was not changed by addition of steroids to the

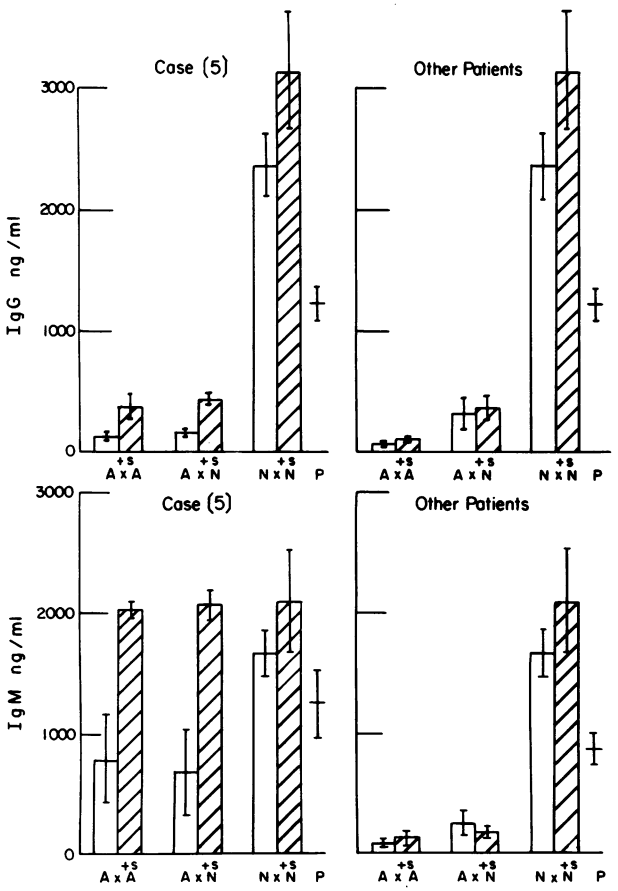

FIGURE 6 The effect of the addition of hydrocortisone succinate (10 μM) to co-cultures of normal and patient MNC (four experiments). A × A, Ig production by 3×10^5 patient cells, A × N, Ig production by an equal mixture of patient and normal MNC. The predicted production, indicated by vertical brackets, is calculated as described in Results. Results are expressed as nanograms per milliliter of Ig, mean±SE. S, hydrocortisone succinate (10 μM). Patient 5 showed a significant increment in IgM production in co-culture assays ($P < 0.005$).

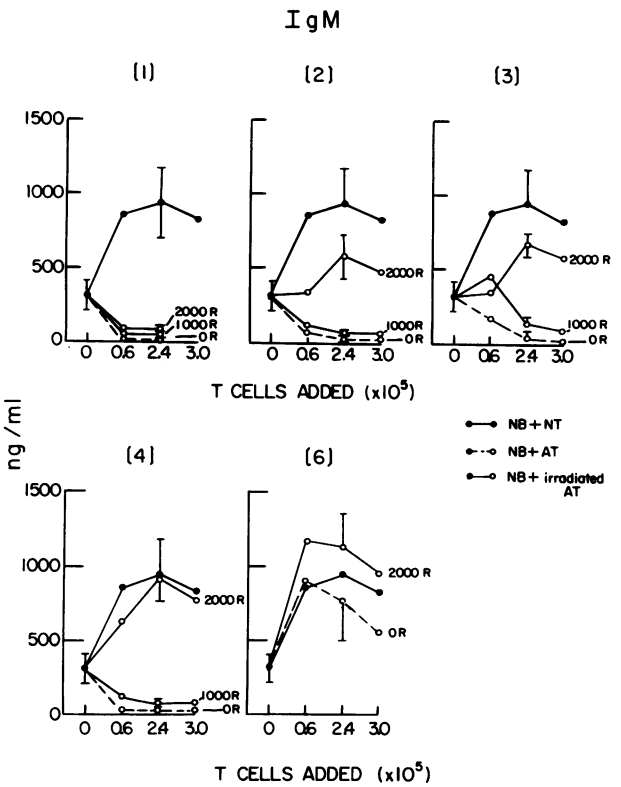

FIGURE 7 The effect of x-ray irradiation of immune-deficient T cells on IgM production. ●, normal cells; ○, immune-deficient cells; R, rad. Results are expressed as mean±SE of three experiments performed in triplicate.

culture. Data illustrating these results are shown in Fig. 6.

Effect of x-ray irradiation. The T cells from immune-deficient patients were irradiated with 1,000 or 2,000 rad. The results of these experiments are shown in Figs. 7 and 8. X-ray irradiation had no effect on the T cells from patient 1. The suppression of normal Ig production was eliminated by 2,000 rad of x-ray irradiation in patients 2–4. As expected, because patient 6 was not suppressive in previous experiments, x-ray irradiation of his T cells had no effect. After 2,000 rad of x-ray irradiation, the T cells from patients 2, 3, and 4 provided essentially normal help. 2,000 rad was more effective than 1,000 rad in the elimination of suppressive cells.

DISCUSSION

Our results support the concept that many patients with combined immunodeficiency and marked hypogammaglobulinemia have circulating mononuclear T cells that suppress the polyclonal activation of normal B cells. These results confirm those originally reported by Waldmann et al. (1). Three of six patients suppressed IgG and IgM synthesis in four different, normal donors. In contrast with this group's universal inhibition of Ig production, however, patient 5 showed selective suppression of IgG production and patients 4 and 6 did not show consistent or significant inhibition of IgG or IgM production in three of four normal donors. Of incidental interest was the fact that in serial studies of several immunodeficient patients a marked variation in proportions of Ia(+) T cells was recorded. Thus, in patient 1 Ia(+) T cells varied between 17 and 50% and in patient 6 between 4 and 46% when studied serially over a 4–6-mo period (Table I). A majority of the patients studied by Siegal et al. (30) and Waldmann and co-workers (1) showed a similar suppression of Ig synthesis. In the latter study the suppression was polyclonal because

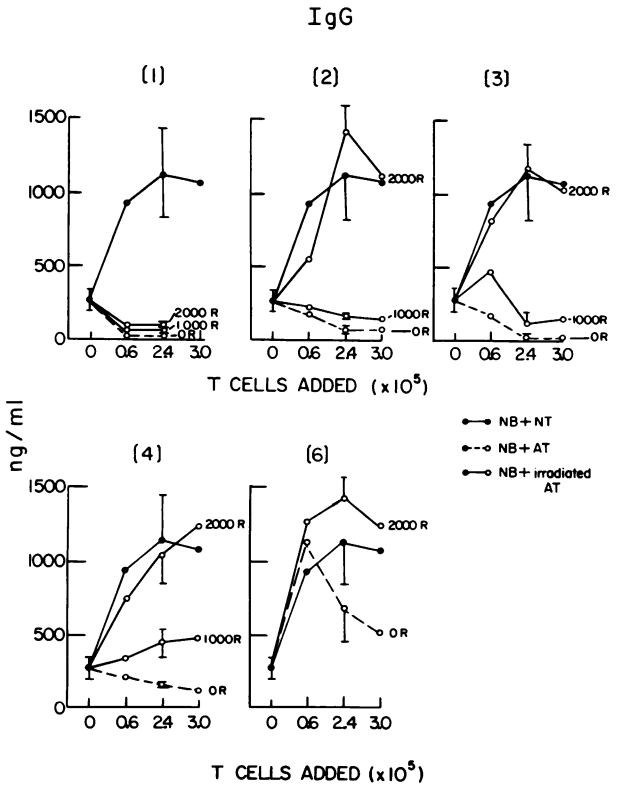

FIGURE 8 The effect of x-ray irradiation of immune-deficient T cells on IgG production. ●, normal cells; ○, immune-deficient cells; R, rad. Results are expressed as mean±SE of three experiments performed in triplicate.

IgG, IgM, and IgA were uniformly depressed. In our study, however, patient 5 showed a selective suppression of IgG alone. The identity of the putative suppressor cell as a T cell is supported by the fact that addition of purified T cells (>95% E rosettes, no monocytes) to normal B cells actually resulted in a significant suppression of base-line IgM and IgG production (for example, patient 2 in Figs. 2 and 3). This was noted, moreover, over a wide ratio of added patient T cells to normal B cells.

Aside from the fact that suppressor T cells exist in these hypogammaglobulinemic patients, two other possibilities for defective patient Ig synthesis must also be considered. First, there may be a lack or functional block in plasma cell precursors and, second, there may be a deficiency of effective helper T cells. With regard to the first possibility, some of our patients, 3 and 4, as well as others previously described (31) have very few or no circulating cells with surface Ig. However, this cannot be the sole explanation for ineffective humoral antibody production because other similar patients, 1, 2, 5, and 6, do show lymphocytes bearing surface Ig. From the data presented here it can be seen that addition of normal T cells did not augment patient Ig production. Curiously, in patient 5 addition of normal T cells did not augment IgM synthesis, but the addition of autologous cells to the in vitro system did. The reason for this discrepancy is not clear because it is generally thought that similarity of the major histocompatibility complex between B and T cells is not essential for in vitro mitogen-stimulated polyclonal Ig synthesis (30). In the case of patient 6, the addition of patient T cells to normal B cells demonstrated a helper effect. A similar addition to autologous B cells showed some augmentation of IgM synthesis, but not of IgG, demonstrating that this patient had normal helper T-cell function but an apparent deficiency of responder B cells. Patient 6 did not exhibit a suppressor effect in co-culture assays with the normal cells used in the experiments shown in Figs. 2 and 3. Thus, at least in this patient, a suppressor T-cell effect was not able to camouflage helper function.

The x-ray irradiation experiments indicated that the suppressor cells were radiosensitive to 2,000 rad. Furthermore, in patients 2–4 such irradiated immune-deficient T cells would function as helpers of normal Ig production. This provides good evidence that the suppression was not simply a lack of helper effect.

One puzzling aspect of the data was the normal amount of IgM (patients 5 and 6, Fig. 2) production when patient B and T cells were cultured in vitro. In other words, no defect in a plasma cell precursor or T-cell immunoregulation was apparent based on such studies. We have no explanation for this incongruity except that there must be undefined defects in the in vivo milieu that would account for decreased serum Ig. One possibility that was not excluded by our data is that there may be increased in vivo Ig catabolism in these patients.

Despite the data in mice (19, 20, 32) and inferential data in man (33) that suppressor immunoregulatory cells may show surface Ia determinants, our co-culture experiments failed to remove the suppressive effect of patient MNC on normal B cells after selective lysis of patient T cells bearing Ia determinants. Similar findings have recently been presented by Fu et al. (22).

Because it has been shown that human T cells with a receptor for IgG (Tγ cells) have a suppressive effect on normal mitogen-stimulated polyclonal Ig synthesis (9), it was logical to examine the possibility that the suppressor T cells in patients with hypogammaglobulinemia might indeed be Tγ cells. Patient Tγ cells were depleted from unfractionated T cells by the incubation of cells with IgG-coated erythrocytes. After such Tγ-cell depletion, patient T cells still significantly suppressed base-line normal Ig production and could

not help patient B cells. These data support the fact that suppressor T cells in all of the hypogammaglobulinemic patients where these experiments were conducted were not invariably Tγ cells. Moretta et al. (10) reported a single patient with thymoma and hypogammaglobulinemia who was able to synthesize Ig after the removal of Tγ cells. The reason for the discrepancy between this latter result and our data is not clear, but may simply represent the fact that some, but not all, suppressor cells are Tγ cells. This possibility is supported by the fact that many of Moretta's hypogammaglobulinemic patients did not show increased proportions of circulating Tγ cells (10). Moreover, there was no relationship between documented in vitro suppression of Ig synthesis and proportions of Tγ cells in our own patients.

Because PGE have been shown to modulate immune function and to be responsible for the impairment of cellular immunity in patients with Hodgkin's disease (34, 35), we examined the possibility that the suppression observed with patient T cells might involve PGE-mediated mechanisms. Accordingly, indomethacin was added to co-cultures between normal cells and patient cells; however, no enhancement of Ig synthesis was observed. These results provide strong evidence that such B-cell suppression was not mediated directly by PGE. Moreover, these data are of particular interest because we have recently demonstrated a temporary reversal of skin test anergy in two of these same patients after a therapeutic trial of indomethacin (36).

17 yr ago Soothill et al. (37) reported a patient with hypogammaglobulinemia who responded to the administration of prednisone with increased serum Ig levels. In support of this observation, our results showed that the suppression of normal IgM production by T cells from one of six patients with hypogammaglobulinemia was reversed in vitro by hydrocortisone. IgG production was not similarly affected. At present this patient has not received a therapeutic trial of corticosteroids because he is fearful of their side effects. Waldmann et al. (38) reported a similar reversal of suppression with corticosteroids in one of their patients. On the basis of these previous observations, despite the negative in vitro data using lymphocyte co-culture techniques in the presence of corticosteroid, one of our patients, 3, underwent a therapeutic trial of 40 mg of prednisone/d for 7 d during a period of time when he was desperately ill with perineal suppuration. This therapeutic trial in his case did not affect either serum Ig levels or the ability of his MNC to produce IgG or IgM in vitro.

Finally, it appears that the depressed serum Ig levels in patients with common variable hypogammaglobulinemia are probably the result of several factors. Various combinations of defective plasma cell precursors coupled with increased numbers of suppressor T cells are instrumental in selective or polyclonal impairment of Ig synthesis. It would appear that the suppressor T cells in combined immunodeficiency patients do not invariably possess Fc-IgG receptors or surface Ia determinants. Furthermore, PGE does not appear to be a causative factor in circulating Ig suppression. The in vitro enhancement of IgM synthesis in one of our patients suggests that intermittent administration of corticosteroids may prove to be clinically useful in some patients where in vitro assays can be undertaken before making a clinical decision as to therapeutic efficacy. Recent data presented by Gelfand et al. (39) suggest that agents such as lithium may modulate or decrease suppressor T-cell activity of lymphocytes in vitro. More precise knowledge of basic regulatory mechanisms that are either overactive or deficient in patients with a spectrum of immune deficiencies may eventually lead to effective therapeutic intervention.

ACKNOWLEDGMENTS

We are grateful to Gregory Howick, Jean Montano, and Kathy Kilpatrick for expert technical assistance.

This work was supported by grants AMAI 13824-10, AL 13433-02, CA 27873 and 13690-09 from the U. S. Public Health Service and in part by a grant from the American Cancer Society.

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