FUNCTIONAL ANALYSIS OF TWO HUMAN T-CELL SUBPOPULATIONS: HELP AND SUPPRESSION OF B-CELL RESPONSES BY T CELLS BEARING RECEPTORS FOR IgM OR IgG*

By LORENZO MORETTA, SUSAN R. WEBB, CARLO E. GROSSI, PETER M. LYDYARD, and MAX D. COOPER

(From the Cellular Immunobiology Unit of the Lurleen B. Wallace Tumor Institute, Departments of Pediatrics and Microbiology, University of Alabama in Birmingham, Birmingham, Alabama 35294)

Thymus-derived (T) lymphocytes of mice can be subdivided according to surface expression of different Ly alloantigens (1–2), presence of different amounts of Thy-1 alloantigen (3), and the presence or absence of Fc receptors for IgG (4–8) on IgM (8a). Heterogeneity of the T-cell populations defined on the basis of qualitative and quantitative variations of surface markers is reflected in differences of various functions of T cells. Surface markers have been used to fractionate T cells and to show that different functions can be attributed to distinct subpopulations (1–3, 8, 9–12).

It has recently been shown that human T-cell subpopulations can be identified and isolated via surface receptors capable of binding either the Fc portion of IgM or the Fc of IgG (14–17). Up to 75% of T lymphocytes in human peripheral blood have receptors for the Fc fragment of IgM ($T_{.M}$)[1], a smaller proportion (<20%) have receptors for IgG ($T_{.G}$), and the remainder lack detectable receptors for either IgM or IgG. Since these two types of Fc receptors are normally present on distinct subpopulations of T cells, it is possible to fractionate T cells with Fc receptors by their capacity to bind IgG or IgM immune complexes, and subsequently to analyze their behavior in in vitro assays (22, 23). We have previously shown that $T_{.M}$ and $T_{.G}$ respond similarly to the T-cell mitogen concanavalin A but differently to phytohemagglutinin, indicating that in the human, as in the mouse, subpopulations of T lymphocytes can be distinguished on the basis of differential mitogen responsiveness (9, 23).

In the present study, we have examined the ability of the two T-cell subpopulations with Fc receptors to provide help for or to suppress the polyclonal B-cell differentiation induced by pokeweed mitogen (PWM). We demonstrate that helper activity is confined to the $T_{.M}$ fraction; $T_{.G}$ cells do not help the induction of either proliferation or differentiation of B cells. Furthermore, after interac-

* Supported by grants CA 16673 and 13148, awarded by the National Cancer Institute, and 5M01-RR32, awarded by the National Institutes of Health.

[1] *Abbreviations used in this paper*: B-cell fraction, nonadherent mononuclear blood cells depleted of SE-RFC; SE, sheep erythrocytes; cIg, cytoplasmic immunoglobulin; FCS, fetal calf serum; IgG-OE, ox erythrocytes coated with rabbit IgG antibody; IgM-OE, ox erythrocytes coated with rabbit IgM antibody; OE, ox erythrocytes; PBL, peripheral blood lymphocytes; PWM, pokeweed mitogen; RFC, rosette-forming cells; sIg, surface immunoglobulin; $T_{.G}$ cells, T lymphocytes with Fc receptors for IgG; $T_{.M}$ cells, T lymphocytes with Fc receptors for IgM.

tion with IgG immune complexes during isolation, $T._G$ cells suppress the generation of plasma cells when added to helper T cells and B cells together with PWM.

Materials and Methods

Cell Separation. Most of the procedures used to purify the various lymphocyte fractions have been described elsewhere (23). Briefly, human peripheral blood lymphocytes (PBL) were isolated on a Ficoll-Hypaque gradient (24) and adherent cells removed by incubation (37°C for 45 min) in plastic flasks. PBL were then pelleted with neuraminidase-treated sheep erythrocytes (SE) and after 45 min the rosetting T cells separated from the nonrosetting B-cell fraction on a second density gradient. The rosette-forming cells (RFC) in the pellet were resuspended and, for further purification, were separated on another Ficoll-Hypaque gradient. This double step isolation procedure gave a suspension containing 99–100% SE-RFC. The rosettes were dissociated by incubation at 37°C for 10–20 min with frequent vortex mixing, and T-cells were separated from the SE on a density gradient previously warmed to 37°C. The B-cell fraction obtained from the interface after the initial density gradient separation of E-rosetting cells was deprived of residual T cells by rosetting again with neuraminidase-treated SE and density gradient separation.

To isolate T lymphocytes which have receptors for IgG, purified T cells were mixed with ox erythrocytes (OE), coated with rabbit IgG antibodies (IgG-OE), pelleted, and incubated on ice for a minimum of 10 min (16). Rosetting cells ($T._G$) were isolated from nonrosetting cells and purified by pelleting through two density gradients. This yielded a cell suspension containing less than 1% nonrosetting cells. Erythrocytes were lysed with ammonium chloride buffer (25).

$T._G$-enriched and $T._G$-depleted cells were cultured overnight in TC 199 medium (Grand Island Biological Co., Grand Island, N. Y.), supplemented with 20% fetal calf serum (FCS), an incubation step necessary for expression of free receptors for IgM. Cells bearing receptors for IgM ($T._M$) present in the $T._G$-depleted fraction were purified on density gradients after rosetting for 1 h on ice with OE coated with rabbit IgM antibodies (IgM-OE) (17). The rosettes were dissociated by vortex agitation and by adding purified human IgM at a concentration of 125 μg/ml in phosphate-buffered saline. $T._M$ cells were then separated from the OE on a density gradient.

B lymphocytes with receptors for IgG were isolated by rosetting the B fraction with IgG-coated OE followed by density gradient separation. The erythrocytes were lysed with ammonium chloride buffer.

Surface Markers on Separated Lymphocyte Populations. Surface Ig-positive cells were identified and enumerated by immunofluorescence by using purified goat antibodies to human $F(ab')_2$ fragments conjugated with tetramethylrhodamine isothiocyanate (molar P/R ratio of 1.5). Lymphocytes binding neuraminidase-treated SE and IgG-coated OE were identified by using techniques described above. The methods for enumeration of cells with surface receptors for human and mouse C3 were used essentially as described previously (26). These assays utilized OE coated with IgM antibodies and mouse or human complement. To assure that rosettes represented binding to C3 receptors rather than to the IgM Fc receptor present on T cells (18), T cells to be assayed were preincubated in RPMI-1640 (Grand Island Biological Co.) containing 1 mg/ml human IgM. This procedure effectively blocks formation of rosettes via the IgM receptor.

Irradiation of Cells. Purified T-cell suspensions received 3,000 rad γ-irradiation from a ^{137}Cs unit at a rate of 700 rad/min. The irradiated cells were >98% viable as judged by trypan blue dye exclusion but were unable to proliferate in response to phytohemagglutinin, concanavalin A, or PWM.

Cell Cultures. Lymphocytes were cultured in flat-bottom Microtest II tissue culture plates (Falcon 3040, Rockville, Md.) at 37°C in an atmosphere of 5% CO_2 in air. The culture medium was RPMI 1640 supplemented with 20% heat-inactivated FCS (International Scientific Industries Inc., Cary, Ill.), 2 mM glutamine (Grand Island Biological Co.) and 50 μg/ml gentamicin (Schering Corporation, Kenilworth, N. J.).

To test helper T-cell function, 5×10^4 cells of the B fraction were mixed with $2.5–100 \times 10^3$ T cells in a final vol of 0.2 ml per well. PWM (Grand Island Biological Co.) was reconstituted with 5 ml distilled water per vial and a 1-μl dose added to each well. The various T-cell fractions and the B-cell fraction were also cultured alone in the presence of PWM at a cell concentration of 10^5/well. Suppressor activity was assessed by adding the various T-cell fractions to a combination of helper T cells and B cells which gave rise to efficient plasma cell differentiation.

In some experiments, the capacity of macrophages to promote T-cell proliferation in response to PWM was tested. Adherent cells were prepared by incubating mononuclear blood cells at 37°C in the microplates at a concentration of 5×10^5 per well. After 30 min, nonadherent cells were removed by extensive washing with medium. This resulted in a diffuse monolayer of adherent cells. T cells and PWM were added to these wells and cultured as described above.

All cultures were harvested at 7 days. Cells were counted by using hemocytometer chambers and the viability assessed by trypan blue exclusion.

Detection of Cytoplasmic Immunoglobulin by Immunofluorescence. Cytocentrifuge preparations of the cultured cells were fixed in ice cold acetic acid (5%) in ethanol (95%), rehydrated in phosphate-buffered saline, and stained at room temperature for 20 min with goat anti-human $F(ab')_2$ fragments or anti-heavy chain antibodies labeled with fluorescein isothiocyanate (molar F/P ratio of 1.7–3.0) or tetramethylrhodamine isothiocyanate (molar P/R ratio of 1.0–1.5) at concentrations of 0.1–0.2 mg/ml. Slides were examined with a Leitz Orthoplan microscope (E. Leitz, Inc., Rockleigh, N. J.) equipped with phase constrast optics and a Ploem epi-illumination system. Filter combinations totally selective for fluorescein and rhodamine, respectively, were used for observation of slides stained for two heavy chain classes (27).

Detection of B and T Cells in Cultures. The percentage of cells with detectable amounts of cytoplasmic immunoglobulin (cIg^+) was determined and the number of plasma cells per well calculated as a function of the number of viable cells. The absolute number of T cells remaining in the cultures at the end of 7 days was calculated from the percentage of SE-RFC and the number of viable cells recovered.

Results

Surface Markers Expressed by the Separated Populations of Peripheral Blood Lymphocytes. Cell fractions were examined for a variety of surface markers distinguishing T and B cells. The results (Table I) indicate that (*a*) the total T-cell fraction contained <1% of cells bearing surface immunoglobulin (sIg) and C3 receptors; (*b*) the purified $T_{.G}$ subpopulations of cells contained fewer than 1% of cells bearing sIg and C3 receptors; (*c*) purified $T_{.M}$ cells were essentially free of cells with sIg or with receptors for IgG; and (*d*) the B-cell fraction was substantially enriched for cells with sIg, receptors for C3, and binding sites for IgG-coated OE.

Interdependence of T and B Cells in the PWM Response. We first confirmed that B lymphocytes do not respond to PWM in the absence of T cells (28, 29). Neither T cells nor B cells increased in number when cultured alone in the presence of the mitogen, nor was there any maturation of B lymphocytes into plasma cells as detected by staining with fluorescent anti-Fab antibodies (Table II). Combination of T- and B-cell fractions in PWM cultures resulted in proliferation of both populations as well as in differentiation of B cells (Fig. 1).

The possibility was considered that the failure of isolated T cells to proliferate might be due to the lack of macrophages, which normally would be present in the B-cell fraction. Prior coating of the tissue culture wells with adherent cells, however, did not promote either the proliferation of T cells in response to PWM or the proliferation and differentiation of separated B cells (Table III). These results suggest a functional interdependence of the T- and B-cell fractions in this model system. Furthermore, this mutual dependence for responses to PWM provided an internal control for the efficiency of the T- and B-cell separation.

Helper Effects of T-Cell Subpopulations on B-Cell Responses. To investigate whether the helper activity in this model system might be restricted to a subpopulation of Fc-positive T cells, we initially separated the $T_{.G}$ from T cells lacking receptors for IgG and cultured these T-cell fractions with a constant

TABLE I

Evaluation of Surface Markers on Separated Populations of Lymphocytes from Human Peripheral Blood

	E-rosettes	sIg$^+$	C3 receptor$^+$	IgG-OE receptor$^+$
	%	%	%	%
PBL	69.6 ± 5.3*	12, 10	9, 11‡	19, 24
B-cell fraction	<1	58, 40	25, 30	49, 58
T	>99 (87)§	0.2, <0.1	<0.5	7, 13
$T._G$	>99 (85)§	0.8, 0.6	0.5, 1	>99
$T._M$	>99 (85)§	<0.1	<0.5	<1

* The mean and standard error was determined from single estimates on PBL from 23 healthy individuals; for the other data entries, results of individual determinations are given. (See Materials and Methods for techniques used for separation of the various lymphocyte fractions and for detection of surface markers).

‡ Both human and mouse complement sources were used with comparable results.

§ Values for cell suspensions incubated for 24 h at 37°C before rosetting again with SE are in brackets.

TABLE II

*Analysis of the Cellular Requirements for PWM-Induced Proliferation of T and B Cells and Differentiation of B Cells**

Population of cells cultured (10^5 cells/culture)	Viable cells recovered/culture ($\times 10^{-3}$)	cIg$^+$ cells/culture‡ ($\times 10^{-3}$)
T + B cells§	380	135
B cells	15	2
T cells	43	<1
T cells with receptors for IgG ($T._G$)	31	<1
T cells without receptors for IgG	36	<1

* The cells were harvested after 7 days in culture with PWM; these results, from a single experiment, were verified in two other experiments.

‡ Fixed cells stained with fluorochrome-labeled anti-F(ab')$_2$ antibodies.

§ Equal numbers (5×10^4) of each cell fraction were added.

number of B cells. The $T._G$-depleted subpopulation was capable of providing help for B-cell differentiation in 7-day cultures containing PWM (Fig. 2a). This result was consistently reproduced in more than 20 experiments. The number of cIg$^+$ cells recovered from these cultures was comparable to that recovered from cultures supplemented with unfractionated T cells (Fig. 1). In other similar experiments (data not shown), the class of Ig present in the cIg$^+$ cells was determined. IgM-, IgG-, and IgA-containing cells were detected in proportions similar to those reported by Keightley et al. who studied T-cell help by using unfractionated T cells in the PWM model system (29).

On the other hand, although $T._G$ cells were capable of a proliferative response to PWM when added to the B-cell-rich fraction, they did not support either proliferation or differentiation of B cells in seven experiments; results of one such experiment are shown in Fig. 2 b.

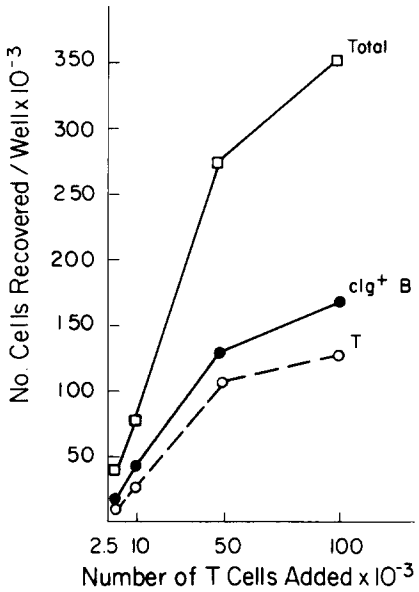

FIG. 1. T-cell requirement for PWM induction of proliferation and differentiation of B cells. Different numbers of T cells purified from PBL were added to microculture wells containing PWM and a constant number of B cells (5×10^4 PBL enriched for B cells by removal of T cells and adherent cells as described in Table I and Materials and Methods). The total number of viable cells, B cells containing cIg by immunofluorescence, and T cells forming rosettes with neuraminidase-treated SE per culture well were enumerated at harvesting after 7 days in culture.

TABLE III

Analysis of the Effect of Adherent Cells on the PWM Responses of Separated T- and B-Cell Fractions

Population of cells cultured (10^5 cells/well)	Viable cells recovered/culture ($\times 10^{-3}$)		cIg^+ cells/culture ($\times 10^{-3}$)	
	Adherent cells added*	No adherent cells added	Adherent cells added*	No adherent cells added
T + B cells	ND	261	ND	124.0
B cells	42	27	1.2	0.5
T cells	21	37	0.1	0.1
T cells with receptors for IgG ($T_{.G}$)	59	36	0.2	0.3
T cells without receptors for IgG	50	42	0.3	0.1

* Culture wells were coated with a diffuse monolayer of adherent cells as described in Materials and Methods.

A large proportion (~70%) of the T cells lacking receptors for IgG had receptors for IgM while the remaining cells had no detectable Fc receptors. To distinguish which of these possessed the helper activity, after removal of $T_{.G}$ cells, we isolated T cells expressing receptors for IgM. As shown in Fig. 3 the recovery of cIg^+ cells from cultures supplemented with purified $T_{.M}$ cells was

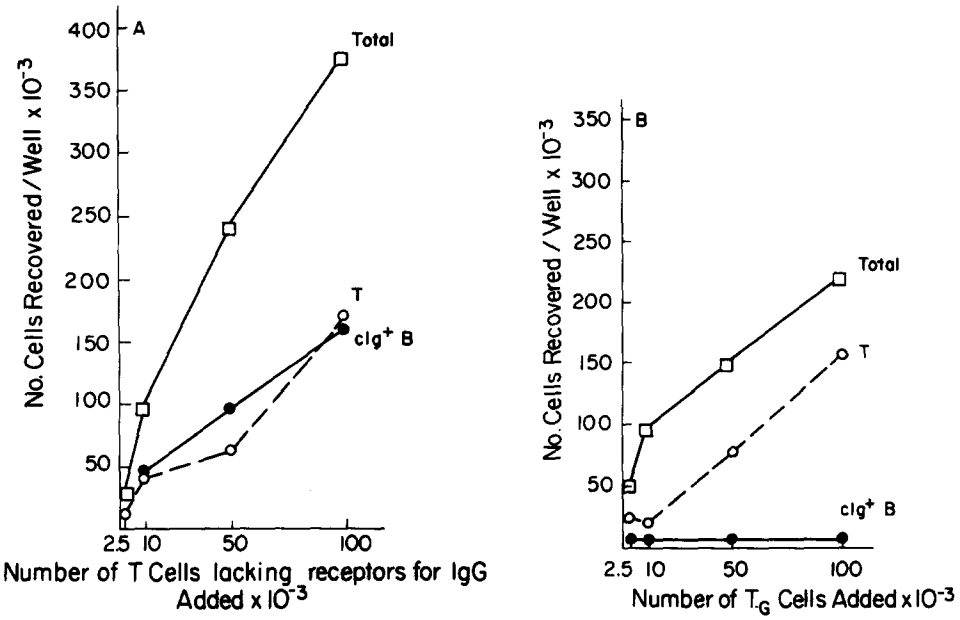

FIG. 2. Role of T-cell subpopulations in PWM induction of proliferation and differentiation of B cells. $T_{.G}$ cells were removed from the total T-cell population by density gradient centrifugation after rosette formation with IgG coated OE as described in Materials and Methods; different numbers of each T-cell fraction were added to a constant number of B cells; and the cells were harvested after 7 days in culture with PWM. (A) T cells depleted of the $T_{.G}$ subpopulation respond with proliferation to PWM and help B cells respond with proliferation and differentiation. (B) $T_{.G}$ cells proliferate in response to PWM but do not help B cells to respond.

even greater than that in cultures supplemented with the total T-cell population, while the removal of $T_{.M}$ cells resulted in a marked decrease in the number of plasma cells recovered from 7-day cultures. In considering this latter observation, which was consistent in three such experiments, it is important to note that because of the fragility of the IgM-OE rosettes, the removal of $T_{.M}$ cells was never complete. Thus the helper activity of the $T_{.M}$-depleted population of T cells was most likely associated with residual $T_{.M}$ cells, although a less efficient help by the Fc-receptor-negative population of T cells cannot be excluded.

Suppressive Effect of $T_{.G}$ Cells on B-Cell Differentiation. In mice, helper activity resides in a subpopulation of T cells lacking receptors for IgG and which is probably distinct from the subpopulation mediating suppression of antibody responses (8). Since $T_{.G}$ cells were incapable of helper function, we investigated whether this subpopulation contained cells capable of suppressor activity. A combination of $T_{.M}$ cells (10^4) and a B-cell enriched fraction (5×10^4) which provided for efficient B-cell responses to PWM without overcrowding the cell cultures was used as an assay for suppressor activity. The effect of adding different numbers of $T_{.G}$, $T_{.M}$, or the total population of T cells on plasma cell differentiation in indicator cultures was determined.

Isolated $T_{.G}$ cells effectively suppressed B-cell differentiation in eight experiments, whereas addition of $T_{.M}$ or unseparated T cells enhanced B-cell differen-

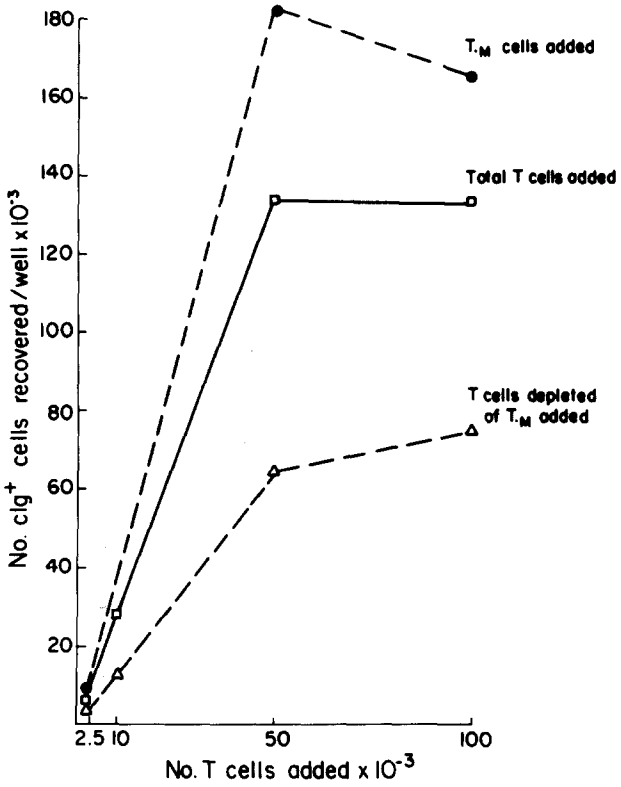

FIG. 3. $T._M$ cells can efficiently help B cells respond to PWM with proliferation and differentiation, whereas T cells depleted of the $T._M$ subpopulation are poor helpers. Different numbers of the total population of T cells, $T._M$ cells or T deprived of $T._M$ cells were added to a constant number of B cells, and clg^+ B cells were enumerated by immunofluorescence after a 7-day culture interval.

tiation (Fig. 4). In other experiments (data not shown), $T._G$ cells also suppressed B-cell differentiation when the ratio of helper $T._M$ cells to B cells was higher (4:5).

In the studies thus far described, the $T._G$ cells were isolated as rosettes with rabbit IgG-coated OE. The erythrocytes were eliminated by lysis. Fragments of the IgG-OE were detected on the surface of freshly isolated $T._G$ cells by phase and immunofluorescence microscopy after staining with fluoresceinated goat antibodies to rabbit IgG. No such fragments were detectable on cells after overnight incubation; in most of the experiments, $T._G$ cells were therefore cultured overnight before use in the differentiation and suppression assays. However, the lack of differentiation in the presence of $T._G$ cells might still be explained by interaction of residual undetectable immune complexes with the Fc receptors present on the B cells. To examine this possibility, cells from a B-enriched fraction were incubated with IgG-coated OE and rosetting cells were isolated by density gradient centrifugation. After lysis of the erythrocytes, these Fc-receptor-positive cells were used as the responding population in a differentiation assay. These B cells were fully capable of differentiating into plasma

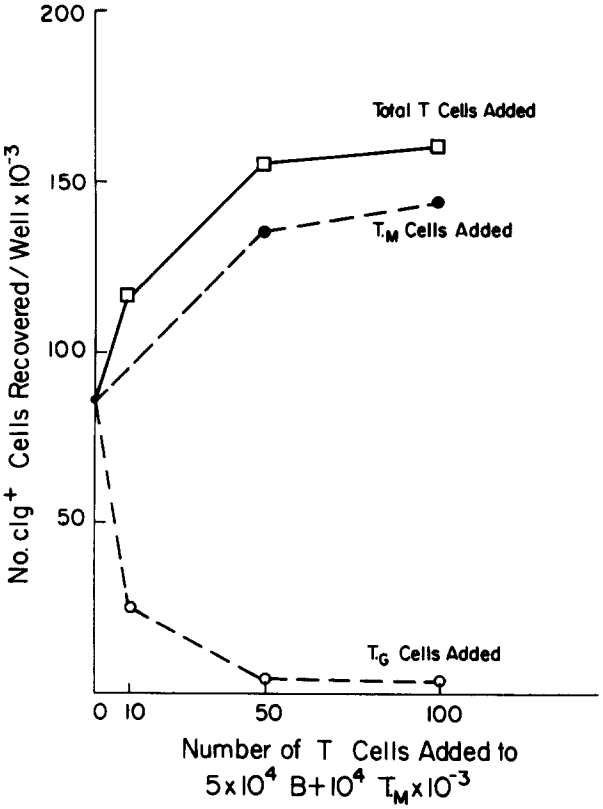

FIG. 4. $T_{.G}$ cells can efficiently suppress the differentiation of B cells in response to PWM plus helper $T_{.M}$ cells. The cultures were harvested at 7 days for enumeration of clg^+ B cells.

cells (Fig. 5). In other experiments, this result was shown not to depend upon whether or not the B cells bearing receptors for IgG were separated by density gradient centrifugation from cells lacking IgG receptors in the original B-enriched fraction. Thus, the suppression of B-cell differentiation by $T_{.G}$ cells was not due to interference by immune complexes.

Requirement for Interaction with Immune Complexes for Suppressor Activity of $T_{.G}$ Cells. Under the in vitro study conditions thus far described, several explanations for the suppressor activity of $T_{.G}$ cells seemed possible. The suppression observed could have been (*a*) acquired in vivo, (*b*) expressed as a consequence of PWM stimulation, (*c*) due to contact with IgG immune complexes, or (*d*) the result of stimulation by both PWM and IgG immune complexes. The first two possibilities were excluded in experiments in which we examined for suppressive activity a $T_{.G}$-enriched fraction obtained by depleting the total T-cell population of $T_{.M}$ cells. 45% of the final suspension were $T_{.G}$ cells in the experiment reported (Fig. 6). A portion of these cells was reacted with IgG-coated OE and then the erythrocytes were lysed; the remaining cells were untreated. The $T_{.G}$-enriched fraction which has been reacted with IgG-OE efficiently suppressed B-cell differentiation, whereas the unreacted fraction did

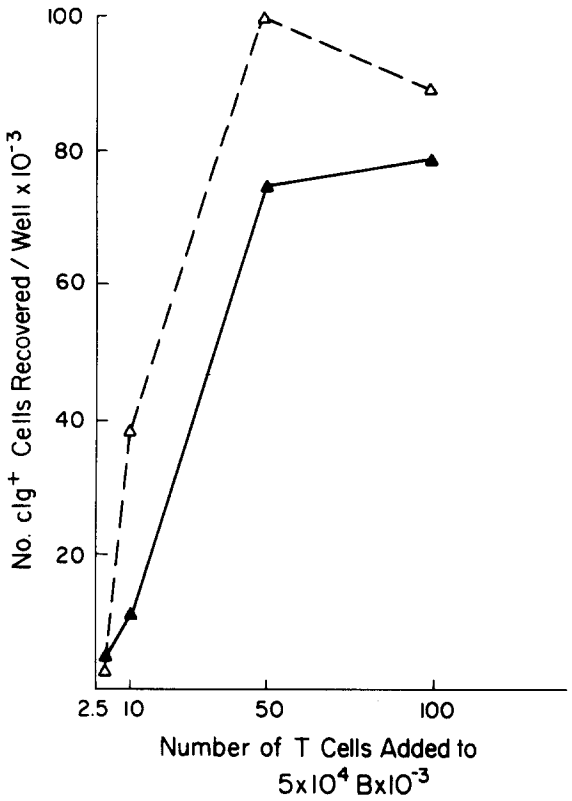

FIG. 5. Binding of IgG immune complexes by B cells does not interfere with their differentiation response to PWM. The helper T cells used in this experiment were depleted of T cells bearing receptors for IgG. These were added either to B cells with receptors for IgG (△- - -△) which were isolated from the B-cell fraction (see Table I and Materials and Methods) or to cells of the B-enriched fraction which were not exposed to IgG-coated erythrocytes (▲—▲).

not. This result was confirmed in another experiment performed in the same way. Thus, the ability of $T_{.G}$ cells to suppress required interaction with immune complexes.

The Effect of Irradiation on the Functional Capabilities of $T_{.M}$ and $T_{.G}$ Cells. To further characterize these populations of T cells, we examined the radiation sensitivity of the helper and suppressor activities in this system. Whereas T cells exposed to 3,000 rad were fully capable of providing B-cell help (Fig. 7, see also reference 29), prior irradiation of $T_{.G}$ cells (3,000 rad) rendered them incapable of suppressing a normal differentiation system (Fig. 8).

Discussion

The differentiation of B lymphocytes into plasma cells after interaction with most antigens or with PWM requires T-cell help. Thus, apart from the polyclonal effect of the mitogen, pokeweed extract appears to mimic the triggering of B cells by T-dependent antigens and, therefore, seems a suitable model for in vitro study of interactions between human T and B cells. In our studies, not only

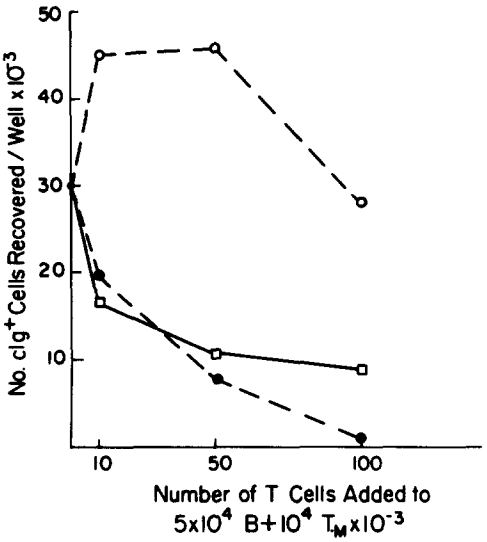

FIG. 6. Requirement for contact with IgG immune complexes for suppressor activity of $T_{.G}$ cells in the PWM response. A T-cell subpopulation enriched for $T_{.G}$ cells by prior depletion of $T_{.M}$ cells were either used without further treatment (O- - -O) or reacted with IgG-coated OE (●- - -●) before comparison of their suppressive activity with a $T_{.G}$ population purified in the usual way (□—□) described in Materials and Methods.

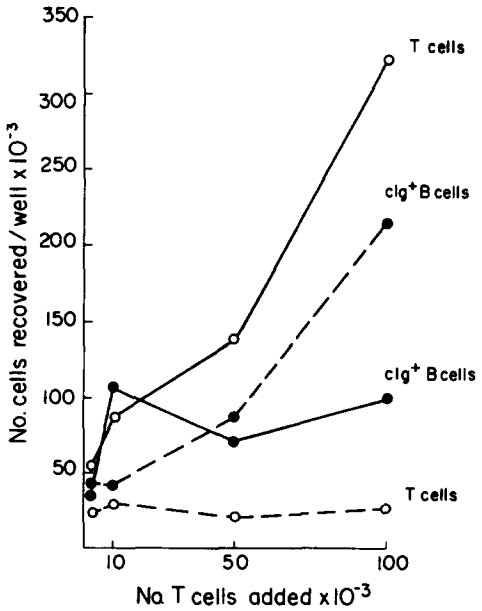

FIG. 7. Effect of irradiation on helper activity in PWM system. Cells of the B fraction were cultured with either untreated T cells (—) or T cells which had previously received 3,000 rad γ-irradiation (- - -). After 7 days the cultures were harvested and evaluated for clg^+ cells and E-rosetting T cells.

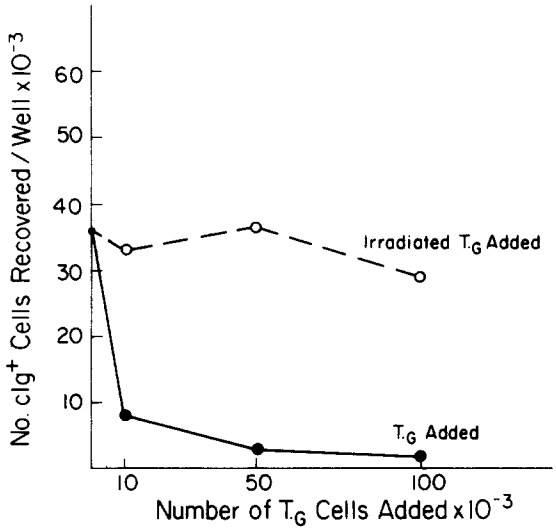

FIG. 8. Effect of irradiation on the suppressor activity of $T_{.G}$ cells. $T_{.G}$ cells were added to a combination of B cells and helper T cells which gave rise after 7 days in culture to the level of cIg^+ cells indicated at the zero point. (●—●) indicates number of cIg^+ cells in cultures supplemented with untreated $T_{.G}$ cells; (O- -O), the number of cIg^+ cells in those cultures supplemented with irradiated $T_{.G}$ cells.

did B cells require T-cell help to respond to PWM, but T-cell proliferation in this system apparently depended on the presence of cells in the B-enriched fraction. Since prior coating of the culture wells with adherent cells did not promote T-cell proliferation in response to PWM, it seems likely that cooperation of cells other than macrophages is required. This apparent requirement could be a function of the dose of mitogen that we used; i.e., purified T cells might proliferate to different doses of PWM. However, over a wide concentration range we noted that higher doses of PWM did not induce any greater degree of thymidine uptake (unpublished observations). The auxiliary cell required is most likely to be a nonadherent sIg-negative cell (30, 31).

Of crucial importance in the interpretation of any functional studies involving separated subpopulations of blood cells is their purity, so we examined this in some detail. The possibility of non-T cells contaminating the $T_{.G}$ fraction seemed especially likely since non-T cells lacking surface immunoglobulin but having receptors for C3 and the Fc of IgG can bind sheep erythrocytes after the treatment of lymphocytes with neuraminidase (32) or after prolonged incubation with sheep erythrocytes under certain conditions (33). Furthermore, a large proportion of cells in the non-T fraction have receptors for IgG and even a small contamination present in the starting population of purified T cells could have been enriched for during the isolation of the $T_{.G}$-cell subpopulation. However, $T_{.G}$ cells lacked plasma membrane-bound Ig and receptors for C3. Elsewhere we have shown that $T_{.G}$ cells lack peroxidase activity, react with an antiserum specific for T cells, are structurally homogeneous both at the light and electron microscopic levels[2], and respond to the T-cell mitogens, phytohemagglutinin,

[2] Grossi, C. E., L. Moretta, A. Zicca, M. C. Mingari, S. R. Webb, P. M. Lydyard, and M. D. Cooper. Morphologic characteristics and distribution of two human T-cell subpopulations. Manuscript in preparation.

and concanavalin A (23). These data together with recent observations of a similar T-cell subpopulation in the mouse (8, 9, 10), strongly support the thesis that the $T._G$-cell fraction constitutes a distinct subpopulation of T lymphocytes that can be isolated without significant contamination with other cell types. Significant contamination of the $T._M$ cells we used seemed highly unlikely because: (*a*) T cells rosetted with neuraminidase-treated SE were passed twice through a density gradient and less than 0.5% of nonrosetting cells could be detected in the total T-cell population purified in this way; (*b*) human non-T cells and $T._G$ cells do not have receptors for IgM detectable by the IgM antibody-coated OE used (23); and (*c*) the $T._M$ fraction of cells was purified from a T-cell population previously depleted of cells bearing receptors for IgG. The purity of the $T._M$ cells was further supported by the absence of surface Ig, C3 receptors, and Fc receptors for IgG as well as by their reactivity with a specific anti-T antiserum,[2] and their morphologic homogeneity.

Analysis of purified T-cell subpopulations in the PWM model system clearly demonstrated that the helper activity for human B-cell responses resides in the $T._M$ subpopulation, and suppressor function in the $T._G$ fraction. Our results are consistent with those of Stout and Herzenberg who found that mouse helper cells in an adoptive transfer system lack Fc receptors for IgG (8). They also substantiate evidence of Cantor and Boyse that helper and suppressor functions are mediated by distinct subpopulations of mouse T cells (1).

The helper activity of human T cells was resistant to irradiation (see also reference 29). The ability of $T._G$ cells to suppress B-cell differentiation, on the other hand, was sensitive to irradiation even when large numbers of $T._G$ cells were added.

One potential function for the receptors of IgM and IgG antibodies is the arming of $T._M$ and $T._G$ cells which may serve to direct their cytotoxic activities against tumor cells and other cell types (34–37).

The functional dichotomy of $T._M$ and $T._G$ cells also tempts one to propose a simple regulatory role for the binding of antibody to T cells, similar to that proposed by Playfair (38) and Gorczynski et al. (39); i.e., binding of IgM antibodies plus antigen to specific $T._M$ cells destined to provide help, exerts a more positive pressure than antigen alone on this T-cell function and thereby on the immune response. Previously McBride and Schierman (40) demonstrated that enhancement of the immune response by anti-carrier antibody required T cells; and Playfair showed that early antibody (presumably IgM) and not late antibody (IgG) was active in the antibody-mediated enhancement of a primary antibody response in mice (41). By using the polyclonal PWM response assay, binding of IgM was not found to be a necessary prerequisite to helper activity. In this model system differentiation of all classes of cytoplasmic Ig^+ cells was promoted regardless of whether or not the T helper cells were exposed to IgM immune complexes.

Binding of IgG immune complexes could serve the antithetical role in this postulated mechanism for regulation of the immune response (42–44) via activation of suppressor T cells which in turn would inhibit the ongoing response (44). The necessity for interaction with immune complexes to induce $T._G$ cells to suppress in the pokeweed system is an important point to consider. Several groups have reported the presence of Fc receptors for IgG on rodent T lympho-

cytes activated by antigen or by allogeneic cells (4–6, 45–47). Our data indicate that $T_{.G}$ cells can bind immune complexes before in vitro activation and that this interaction can promote the functional activation of these cells.

Hyperactivity of suppressor T cells (48) has been demonstrated in vitro in several disorders of the immune system in man (49). By using the PWM response assay, the presence of T-cell-mediated suppressor function has been demonstrated in patients with common variable hypogammaglobulinemia (50, 51), although conflicting data have been obtained regarding this issue (52–54). Enhanced suppressor activity has also been reported in individuals with thymoma and immunodeficiency, isolated IgA deficiency, X-linked infantile agammaglobulinemia, and chronic fungal infections (49, 51, 55). Nonadherent suppressor cells have also been observed in patients with Hodgkin's disease (56). We have shown that circulating $T_{.G}$ cells may be present in increased proportions, often accompanied by an absolute decrease in numbers of circulating $T_{.M}$ cells, in individuals with thymoma and hypogammaglobulinemia, Hodgkin's disease, and chronic fungal infection, and occasionally in patients with common variable hypogammaglobulinemia, IgA deficiency, and X-linked agammaglobulinemia (22, 57).[3] Moreover, we noted that removal of $T_{.G}$ cells eliminated the suppressor activity exhibited by peripheral blood lymphocytes from an immunodeficient individual with thymoma.[3] Increased proportions of $T_{.G}$ cells exist in cord blood,[2] where suppressor activity has also been noted (58–60). Whether or not naturally occurring IgG immune complexes or IgG aggregates administered during replacement gammaglobulin therapy of immunodeficient patients plays a role in the activation of suppresor $T_{.G}$ cells remains to be determined, but our present results suggest that this is a distinct possibility.

Summary

Subpopulations of thymus-derived T lymphocytes bearing receptors for either IgM or IgG molecules were isolated from human peripheral blood. Those with receptors for IgM ($T_{.M}$) provided help in a cell dose-dependent fashion for the pokeweed mitogen-induced differentiation of B lymphocytes in vitro, whereas cells with receptors for IgG ($T_{.G}$) did not. $T_{.G}$ cells, on the other hand, efficiently suppressed the differentiation and proliferation of B cells in the pokeweed system in the presence of helper $T_{.M}$ cells. This suppressive activity of $T_{.G}$ cells required prior interaction of the $T_{.G}$ cells with immune complexes. The helper activity of $T_{.M}$ cells was relatively radioresistant while the suppressor activity of $T_{.G}$ cells was radiosensitive. The results indicate that helper and suppressor functions of human T lymphocytes in this model system are mediated by different subpopulations of T cells which can be distinguished by their ability to bind IgM or IgG immune complexes, respectively.

We would like to thank our colleagues for the helpful discussions, and Doctors A. R. Lawton and R. D. Stout for critical reading of the manuscript.

Received for publication 23 March 1977.

---

[3] Moretta, L., M. C. Mingari, S. R. Webb, E. P. Pearl, P. M. Lydyard, C. E. Grossi, A. R. Lawton, and M. D. Cooper. Imbalances in T-cell subpopulations associated with immunodeficiency and autoimmune syndromes. Manuscript submitted for publication.

References

1. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. *J. Exp. Med.* 141:1376.
2. Hirst, J. A., P. C. L. Beverley, P. Kisielow, M. K. Hoffman, and H. E. Oettgen. 1975. Ly antigens: markers of T cell function on mouse spleen cells. *J. Immunol.* 115:1555.
3. Cantor, H., E. Simpson, V. L. Sato, C. G. Fathmann, and L. A. Herzenberg. 1975. Characterization of subpopulations of T lymphocytes. I. Separation and functional studies of peripheral T-cell binding different amounts of fluorescent anti thy 1.2 (Theta) antibody using a fluorescence-activated cell sorter (FACS). *Cell. Immunol.* 15:180.
4. Lee, S. T., and F. Paraskevas. 1972. Cell surface-associated gammaglobulins in lymphocytes. IV. Lack of detection of surface γ_G globulin by T-cell during primary response. *J. Immunol.* 109:1262.
5. Yoshida, T. O., and B. Andersson. 1972. Evidence for a receptor recognizing antigen complexed immunoglobulin on the surface of activated mouse thymus lymphocytes. *Scand. J. Immunol.* 1:401.
6. Soteriades-Vlaches, C., M. I. Gyöngyössy, and J. H. L. Playfair. 1974. Rosette formation by mouse lymphocytes: III. Receptors for immunoglobulin on normal and activated T cells. *Clin. Exp. Immunol.* 18:187.
7. Anderson, C. L., and H. M. Grey. 1974. Receptors for aggregated IgG on mouse lymphocytes. Their presence on thymocytes, thymus-derived and bone marrow-derived lymphocytes. *J. Exp. Med.* 139:1175.
8. Stout, R. D., and L. A. Herzenberg. 1975. The Fc receptor on thymus-derived lymphocytes. I. Detection of a subpopulation of murine T lymphocytes bearing the Fc receptor. *J. Exp. Med.* 142:611.

8a. Lamon, E. W., B. Andersson, H. D. Whitten, M. M. Hurst, and V. Ghanta. 1976. IgM complex receptors on subpopulations of murine lymphocytes. *J. Immunol.* 116:1199.

9. Stout, R. D., and L. A. Herzenberg. 1975. The Fc receptor on thymus-derived lymphocytes. II. Mitogen responsiveness of T lymphocytes bearing the Fc receptor. *J. Exp. Med.* 142:1041.
10. Stout, R. D., S. D. Waksal, and L. A. Herzenberg. 1976. The Fc receptor on thymus-derived lymphocytes. III. Mixed lymphocyte reactivity and cell-mediated lympholytic activity of Fc^- and Fc^+ T lymphocytes. *J. Exp. Med.* 144:54.
11. Stout, R. D., D. B. Murphy, H. O. McDevitt, and L. A. Herzenberg. 1977. The Fc receptor on thymus-derived lymphocytes. IV. Inhibition of binding of antigen-antibody complexes to Fc receptor-positive T cells by anti-Ia sera. *J. Exp. Med.* 145:187.
12. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Ly^+ cells in the generation of killer activity. *J. Exp. Med.* 141:1390.
13. Niederhuber, J. E., J. A. Frelinger, M. S. Dine, P. Shoffner, E. Dugan, and D. C. Shreffler. 1976. Effects of anti-Ia sera on mitogenic responses. II. Differential expression of the Ia marker on phytohemmagglutinin and concanavalin A-reactive T cells. *J. Exp. Med.* 143:372.
14. Dickler, H. B., N. F. Adkinson, and W. D. Terry. 1974. Evidence for individual human peripheral blood lymphocytes bearing both B and T cell markers. *Nature (Lond.).* 247:213.
15. Brown, G., and M. F. Greaves. 1974. Cell surface markers for human T and B lymphocytes. *Eur. J. Immunol.* 4:302.
16. Ferrarini, M., L. Moretta, R. Abrile, and M. L. Durante. 1975. Receptors for IgG

molecules on human lymphocytes forming spontaneous rosettes with sheep red cells. *Eur. J. Immunol.* 5:70.

17. Moretta, L., M. Ferrarini, M. L. Durante, and M. C. Mingari. 1975. Expression of a receptor for IgM by human T cells *in vitro*. *Eur. J. Immunol.* 5:565.
18. McConnell, I., and C. M. Hurd. 1976. Lymphocyte receptors. II. Receptors for rabbit IgM on human T lymphocytes. *Immunology.* 30:835.
19. Gmelig-Meyling, F., M. Van der Ham, and R. E. Ballieux. 1976. Binding of IgM by human T lymphocytes. *Scand. J. Immunol.* 5:487.
20. Ferrarini, M., L. Moretta, M. C. Mingari, P. Tonda, and B. Pernis. 1976. Human T cell receptor for IgM: specificity for the pentameric Fc fragment. *Eur. J. Immunol.* 6:520.
21. Conradie, J. D., and M. O. Bubb. 1977. Cμ4 domain of IgM has cytophilic activity for human lymphocytes. *Nature (Lond.).* 265:60.
22. Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydyard, and M. D. Cooper. 1976. Functional analysis of two subpopulations of human T cells and their distribution in immunodeficient patients. *Clin. Res.* 24:448A.
23. Moretta, L., M. Ferrarini, M. C. Mingari, A. Moretta, and S. R. Webb. 1976. Subpopulations of human T cells identified by receptors for immunoglobulins and mitogen responsiveness. *J. Immunol.* 117:2171.
24. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* 21(Suppl. 97):77.
25. Boyle, W. 1968. An extension of the ^{51}Cr-release assay for the estimation of mouse cytotoxins. *Transplantation (Baltimore).* 6:761.
26. Ferrarini, M., G. P. Tonda, A. Risso, and G. Viale. 1975. Lymphocyte membrane receptors in human lymphoid leukemias. *Eur. J. Immunol.* 5:89.
27. Kearney, J. F., and A. R. Lawton. 1975. B lymphocyte differentiation induced by lipopolysaccharide. I. Generation of cells synthesizing four major immunoglobulin classes. *J. Immunol.* 115:671.
28. Janossy, G., and M. Greaves. 1975. Functional analysis of murine and human B lymphocyte subsets. *Transplant. Rev.* 24:177.
29. Keightley, R. G., M. D. Cooper, and A. R. Lawton. 1976. The T-cell dependence of B cell differentiation induced by PWM. *J. Immunol.* 117:1538.
30. Kreth, H. W., and L. A. Herzenberg. 1974. Fluorescence-activated cell sorting of human T and B lymphocytes. I. Direct evidence that lymphocytes with a high density of membrane-bound immunoglobulin are precursors of plasmacytes. *Cell. Immunol.* 12:396.
31. Chess, L., R. P. MacDermott, and S. F. Schlossman. 1974. Immunologic functions of isolated human lymphocyte subpopulations. I. Quantitative isolation of human T and B cells and response to mitogens. *J. Immunol.* 113:1113.
32. Bentwich, Z., S. D. Douglas, E. S. Kutelsky, and H. G. Kunkel. 1973. Sheep red cell binding to human lymphocytes treated with neuraminidase; enhancement of T cell binding and identification of a subpopulation of B cells. *J. Exp. Med.* 137:1532.
33. Winchester, R. J., S. M. Fu, T. Hoffman, and H. G. Kunkel. 1975. IgG on lymphocyte surfaces; technical problems and the significance of a third cell population. *J. Immunol.* 114:1210.
34. Lamon, E. W., M. W. Shaw, S. Goodson, B. Lidin, A. S. Walia, and E. W. Fuson. 1977. Antibody-dependent cell-mediated cytotoxicity in the Moloney sarcoma virus system: differential activity of IgG and IgM with different subpopulations of lymphocytes. *J. Exp. Med.* 145:302.
35. Wahlin, B., H. Perlmann, and P. Perlmann. 1976. Analysis by a plaque assay of IgG- or IgM-dependent cytolytic lymphocytes in human blood. *J. Exp. Med.* 144:1375.

36. Fuson, E. W., and E. W. Lamon. 1977. IgM-induced cell mediated cytotoxicity with antibody and effector cells of human origin. *J. Immunol.* In press.
37. Saal, J. G., E. P. Rieber, M. Hadam, and G. Riethmuller. 1977. Lymphocytes with T-cell markers cooperate with IgG antibodies in the lysis of human tumor cells. *Nature (Lond.).* **265**:158.
38. Playfair, J. H. L. 1976. The role of antibody in T-cell responses. *Clin. Exp. Immunol.* **17**:1.
39. Gorczynski, R., S. Koutiainer, N. A. Mitchison, and R. E. Tigelaar. 1974. Antigen-antibody complexes as blocking factors on the T lymphocyte surface. *In* Cellular Selection and Regulation in the Immune Response. E. M. Edelman, editor. Raven Press, New York. **29**:143.
40. McBride, R. A., and L. W. Schierman. 1973. Thymus dependency of antibody-mediated helper effect. *J. Immunol.* **110**:1710.
41. Playfair, J. H. L., S. Marshall-Clarke, and L. Hudson. 1974. Cooperation by mouse T lymphocytes: The role of antibody in T-cell specificity. *Eur. J. Immunol.* **4**:54.
42. Uhr, J. W., and G. Möller. 1968. Regulatory effect of antibody on the immune response. *Adv. Immunol.* **8**:81.
43. Sinclair, N. R., St. C. 1969. Regulation of the immune response. I. Reduction in ability of specific antibody to inhibit long-lasting immunological priming after removal of the Fc fragment. *J. Exp. Med.* **129**:1183.
44. Gershon, R. K., M. B. Mokyr, and M. S. Mitchell. 1974. Activation of suppressor T cells by tumor cells and specific antibody. *Nature (Lond.).* **250**:594.
45. Greaves, M., G. Janossy, and M. Doenhoff. 1974. Selective triggering of human T and B lymphocytes in vitro by polyclonal mitogens. *J. Exp. Med.* **140**:1.
46. Van Boxel, J. A., and D. L. Rosenstreich. 1974. Binding of aggregated γ-globulin to activated T lymphocytes in the guinea pig. *J. Exp. Med.* **139**:1002.
47. Goldschneider, I., and R. B. Cogen. 1973. Immunoglobulin molecules on the surface of activated T lymphocytes in the rat. *J. Exp. Med.* **138**:163.
48. Pierce, C. W., and J. A. Kapp. 1976. Regulation of immune responses by suppressor T cells. *In* Contemporary Topics in Immunobiology V. W. O. Weigle, editor. Plenum Publishing Corporation, New York. 91.
49. Waldmann, T. A., and S. Broder. 1977. Suppressor cells in the regulation of the immune response. *In* Progress in Clinical Immunology. R. S. Schwartz, editor. Grune & Stratton, Inc., New York. 155.
50. Waldmann, T. A., S. Broder, R. M. Blaese, M. Durm, M. Blackman, and W. Strober. 1974. Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinemia. *Lancet.* **2**:609.
51. Siegal, F. P., Siegal, M., and R. A. Good. 1976. Suppression of B-cell differentiation by leukocytes from hypogammaglobulinemic patients. *J. Clin. Invest.* **58**:109.
52. Geha, R. S., E. Schneeberger, E. Merler, and F. S. Rosen. 1974. Heterogeneity of "acquired" or common variable agammaglobulinemia. *N. Engl. J. Med.* **291**:1.
53. Cooper, M. D., R. G. Keightley, and A. R. Lawton. 1975. Defective T and B cells in primary immunodeficiencies. *In* Membrane Receptors of Lymphocytes. Seligmann, M., J. L. Preud'homme, and F. M. Kourilsky, editors. North-Holland Publishing Company, Amsterdam. 431.
54. De La Concha, E. G., G. Oldham, A. D. B. Webster, G. L. Asherson, and T. A. E. Platts-Mills. 1977. Quantitative measurements of T- and B-cell function in "variable" primary hypogammaglobulinemia: evidence for a consistent B-cell defect. *Clin. Exp. Immunol.* **27**:208.
55. Stobo, I. D., S. Paul, R. E. Van Scoy, and P. E. Hermans. 1976. Suppressor thymus-derived lymphocytes in fungal infection. *J. Clin. Invest.* **57**:319.

56. Twomey, J. J., A. H. Laughter, S. Farrow, and C. C. Douglass. 1975. Hodgkin's disease: An immuno-depleting and immunosuppressive disorder. *J. Clin. Invest.* 56:467.
57. Cooper, M. D., L. Moretta, S. R. Webb, E. R. Pearl, A. J. Okos, and A. R. Lawton. Diversity of defects in human B-cell differentiation. *In* International Symposium of Immunopathology. P. Miescher, editor. Schwabe & Co., Basel, Switzerland. In press.
58. Olding, L. B., and M. B. A. Oldstone. 1976. Thymus derived peripheral lymphocytes from human newborns inhibit division of their mother's lymphocytes. *J. Immunol.* 116:682.
59. Lawler, S. D., E. O. Ukaejiofo, and B. R. Reeves. 1975. Interaction of maternal and neonatal cells in mixed lymphocyte culture. *Lancet.* ii:1185.
60. Hayward, A. R., and A. R. Lawton. Interactions of human neonatal T and B lymphocytes and evidence for functional immaturity. *In* Regulatory Mechanisms in Lymphocyte Activation. Proceedings of the 11th Leukocyte Culture Conference. D. O. Lucas, editor. Academic Press, Inc., New York. In press.