The Journal of Immunology

RESEARCH ARTICLE | FEBRUARY 01 1980

Functional properties of human T cells bearing Fc receptors for IgA. I. Mitogen responsiveness, mixed lymphocyte culture reactivity, and helper activity for B cell immunoglobulin production. FREE

L G Lum; ... et. al

J Immunol (1980) 124 (2): 702–707.
https://doi.org/10.4049/jimmunol.124.2.702

0022-1767/80/1242-0702$00.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 124, No. 2, February 1980
Printed in U.S.A.

FUNCTIONAL PROPERTIES OF HUMAN T CELLS BEARING Fc RECEPTORS FOR IgA

I. Mitogen Responsiveness, Mixed Lymphocyte Culture Reactivity, and Helper Activity for B Cell Immunoglobulin Production

LAWRENCE G. LUM, ETTY BENVENISTE, AND R. MICHAEL BLAESE[1]

From the Cellular Immunology Section, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

T lymphocytes bearing Fc-IgA receptors were studied for proliferative responses to mitogens and allogeneic cells, and for their ability to provide help for B cell activation in pokeweed mitogen-driven immunoglobulin production. These responses were compared with those lymphocytes bearing Fc-IgG and Fc-IgM receptors.

T_A cells were found to be generally less responsive in all functional assays than T_M or T_G cells. Their proliferative responses to PHA and PWM averaged 5 to 20% of the responses seen with the other T cell subsets. The T_A cell response to mitogen was more significantly augmented by the addition of irradiated adherent accessory cells than were responses of the other T cell subpopulations. In addition, the peak response of the T_A population to PHA and PWM occurred significantly later than the peak response of unseparated T cells or the other T cell subsets. T_A proliferative responses to allogeneic stimulator cells in mixed lymphocyte cultures averaged approximately one-third of the response of T_M cells, and one-half the response given by T_G cells.

Each of the T cell subsets was found to provide help for B cell activation by pokeweed mitogen, although again the T_A cells were less active than the other T cell subsets in helping immunoglobulin production *in vitro*. Helper activity for each class of immunoglobulin was present in each of the T cell subsets, regardless of the specificity of the Fc receptor expressed on the T cell.

Since the description of Fc-IgG and Fc-IgM receptors on human T cells (1–5), the functional characteristics of Fc-IgG (T_G)[2] and Fc-IgM (T_M) receptor-bearing human T lymphocytes have been studied extensively (6–15).

Distinct functional differences between T_G and T_M cells have been described in assays measuring proliferation to mitogens (10), helper and suppressor activity for B cell differentiation (11, 12), and cytotoxic capacity in antibody-dependent cellular cytotoxicity (ADCC) (14) and mitogen-induced cellular cytotoxicity (MICC) (15).

Recently, we described a subpopulation of human T cells bearing Fc-IgA receptors (16). From 2 to 18% (mean 6.7%) of purified human T lymphocytes express Fc-IgA receptors after overnight culture. In this study, we investigated the function of T cells bearing Fc-IgA receptors (T_A) in mitogen responsiveness to PHA and pokeweed mitogen (PWM), responses to allogeneic cells in mixed lymphocyte culture, and helper cell function in PWM-driven immunoglobulin production.

MATERIALS AND METHODS

Purification of T lymphocyte populations. Human peripheral blood mononuclear cells were prepared from 500 ml of heparinized whole blood by Ficoll-Hypaque (F/H) density gradient centrifugation. Monocytes were depleted by plastic adherence as described previously (10–15). The nonadherent mononuclear cells were rosetted with AET (see abbreviations) modified sheep erythrocytes ($SRBC^{AET}$) as described previously (16, 17). In brief, the monocyte-depleted mononuclear cells were mixed with $SRBC^{AET}$ at a ratio of 150 SRBC to 1 lymphocyte in balanced salt solution (BSS) containing 20% fetal calf serum (FCS), pelleted at 800 × G for 10 min, incubated in ice H_2O for 1 hr, resuspended gently, layered over F/H, and centrifuged for 20 min at 800 × G and 7 min at 1800 × G. The E rosette-positive pellet was lysed with NH_4Cl Tris lysing buffer and washed three times in BSS containing 20% FCS and cultured in medium 199 supplemented with 20% FCS, penicillin-streptomycin, 4 mM glutamine, and 10 mM HEPES (see abbreviations) buffer unless otherwise mentioned. All subpopulations were maintained in supplemented medium 199 at a cell concentration of 2×10^6 cells/ml until they were harvested just before the functional assays.

Received for publication August 6, 1979.
Accepted for publication October 8, 1979.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Please address correspondence to: R. Michael Blaese, M.D., Cellular Immunology, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bldg. 10, Room 4N108, Bethesda, Maryland 20205.

[2] Abbreviations used in this paper: ADCC, antibody-dependent cellular cytotoxicity; AET, 2-amino-isothiouronium bromide hydrobromide; BSS, balanced salt solution; EAIgA, TNP-modified ox erythrocytes coated with MOPC-315 IgA anti-TNP; EAIgG, ox erythrocytes coated with IgG; EAIgM, ox erythrocytes coated with IgM; F/H, Ficoll-Hypaque; HEPES, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid; MICC, mitogen-induced cellular cytotoxicity; Mϕ, adherent accessory cells enriched from monocytes; ORBC, ox erythrocytes; $ORBC^{TNP}$, TNP-modified ox erythrocytes; PWM, pokeweed mitogen; $SRBC^{AET}$, AET-modified SRBC; sIg^+, surface immunoglobulin-positive cells; T, lymphocytes that form rosettes with sheep erythrocytes; T_A, T cells bearing Fc-IgA receptors; T_G, T cells bearing Fc-IgG receptors; T_M, T cells bearing Fc-IgM receptors; RHPA, reverse hemolytic plaque assay.

Purification of adherent accessory cells. Autologous adherent accessory cells were obtained by plastic adherence of F/H-purified mononuclear cells. The mononuclear cells were incubated 1 hr at 37°C in BSS containing Ca^{++}, Mg^{++}, and 20% FCS to allow the monocytes to adhere. After the nonadherent cells were decanted, the flasks were rinsed briskly four times with warm BSS containing Mg^{++}, Ca^{++}, and 20% FCS. The adherent accessory cells were cultured in the flasks in supplemented medium 199 for 14 to 24 hr at 37°C. The adherent accessory cells were then collected by incubation of the culture flasks on ice for 30 min in the presence of Ca^{++}-, Mg^{++}-free BSS. Viability of the recovered cells was greater than 95% by trypan blue exclusion. These autologous adherent accessory cells were irradiated with 3000 R before use.

Separation of T cell subsets. Preparation of the T cell subsets was performed by using antibody-coated ox erythrocytes (ORBC) prepared as previously described (16). TNP-modified ORBC ($ORBC^{TNP}$) or ORBC not coated with antibody always gave less than 0.5% rosettes.

In brief, T_G cells were prepared from freshly prepared T cells by rosetting T cells with ORBC coated with IgG (EAIgG) indicator cells. The indicator cells and T cells were mixed at a ratio of 100:1, pelleted, incubated at 4°C for at least 20 min, resuspended, layered over a cold F/H gradient, and centrifuged for 20 min at 800 × G and 7 min at 1800 × G at 4°C. The T_G cell-enriched pellet (85 to 97% rosette positive) was lysed with NH_4Cl Tris lysing buffer, washed three times in BSS containing 20% FCS, placed into medium 199, and stored at 4°C. The interface containing T non_G cells was washed three times in BSS containing 20% FCS and placed into culture at 2×10^6 cells/ml at 37°C in a CO_2 incubator.

The T_M cells were positively selected from the T non_G population after overnight incubation at 37°C by rosetting with ORBC coated with IgM (EAIgM) indicator cells. The T_M cells were separated by a cold F/H density gradient centrifugation as outlined earlier. The rosette-enriched pellets contained from 85 to 95% rosette-positive cells.

T_A cells were prepared by rosetting unfractionated T cells with EAIgA (see abbreviations) indicator cells. The indicator cell to lymphocyte ratio was 50:1. The rosette mixture was incubated at 4°C for at least 20 min, resuspended, layered over cold F/H, and centrifuged 20 min at 800 × G and 7 min at 1800 × G. The T_A-enriched pellets (94 to 97% rosette positive) were treated in the same manner described for the other subpopulations.

Cell viability. Immediately before initiation of the various functional assays, the T lymphocytes and the subpopulations of T cells were counted and adjusted to the proper concentrations in the appropriate media. Viability by trypan blue exclusion was greater than 95% in all of the experiments.

Lymphocyte blast transformations. The various cell populations were adjusted to 1×10^6 cells/ml in RPMI 1640 medium containing 10% autologous or AB plasma. 1.5×10^5 cells in 0.15 ml were placed in flat-bottom wells of Linbro microtiter plates. Fifty microliters of PHA (Wellcome, Beckenham, England), or PWM (Gibco, Grand Island, N.Y.) were added in the doses indicated. The cultures were performed in triplicate for the number of days indicated, pulsed with tritiated thymidine (specific activity of 6.7 Ci/μm from New England Nuclear, Boston, Mass.) for 4.5 hr, harvested on a Mash II Harvester (Microbiological Associates, Rockville, Md.), and counted on a liquid scintillation counter.

To study the effects of adherent accessory cells on the ability of the various T cell subpopulations to proliferate to mitogens, 50 μl of irradiated accessory cells containing 0.3×10^5 esterase-positive cells (18) were added to 1.5×10^5 T cells or T cell subsets in each experiment. The accessory cells received 3000 R of irradiation.

Mixed lymphocyte cultures. Responder cells (1×10^5) were placed in flat-bottom wells of Linbro plates with 1×10^5 allogeneic stimulator cells that had received 3000 R. The mixed lymphocyte cultures were pulsed, harvested, and counted after 6 days of culture in the same manner as the mitogen-stimulated cultures.

PWM-induced immunoglobulin production. The various T cell subpopulations were added to B cells in the presence of PWM, and the number of immunoglobulin-secreting cells in such cultures after 6 or 7 days of incubation was determined by a reverse hemolytic plaque assay (RHPA) as described previously (19).

From 3.0×10^3 to 10^6 T cells or T cell subpopulations were added to 2×10^5 cells from the population enriched in B cells (non-T cell populations depleted twice of E+ positive cell by $SRBC^{AET}$ rosetting). All cultures were done for 6 or 7 days in the presence of 1.25 μg PWM or media as a control in 16-mm culture wells (Costar) or 15-ml Falcon plastic conical tubes. After 6 days of culture at 37°C in an humidified atmosphere containing 5% CO_2, the cultures were harvested and washed three times in BSS. The number of immunoglobulin-secreting cells was determined in duplicate. The results are reported as plaque-forming cells per culture. For total immunoglobulin production, a polyvalent antiserum with specificity for γ, μ, α, κ, and λ was employed as a developing reagent in the RHPA. In the determination of class-specific immunoglobulin production, monospecific antiserum with specificity for α, γ, or μ was employed.

RESULTS

Proliferative responses to PHA. The responses of T cells and the T cell subsets to varying doses of PHA after 3 days in culture are shown in Figure 1. In this representative experiment, the optimal responses of all cell populations tested occurred at PHA concentrations from 0.25 and 0.5 μg PHA/culture. In agreement with the observations of Moretta *et al.* (10), we have consistently observed that T_G cells respond relatively better than T_M cells at lower PHA concentrations, although this is somewhat obscured by the logarithmic method of data presentation used in Figure 1. It is apparent from this figure that although the T_A subset responds to PHA, the magnitude of this response is significantly less than that of T cells, T_G cells, or T_M cells. Table I shows five experiments comparing the responses of these T cell subpopulations with 0.5 μg PHA. These experiments confirm the relatively impaired response of the T_A subpopulation to this mitogen.

Table II demonstrates that unfractionated T cells show peak proliferation at day 3 of culture and that this response is diminished to about one-half by day 5. The response of the T_M subset was relatively constant between days 3 and 5, and the response of the T_G subset tended to fall off somewhat between days 3 and 5. By marked contrast, the response of the T_A subset increased 4-fold between days 3 and 5 of culture.

Proliferative response to PWM. Figure 2 shows the responses at 6 days of the T cell subsets to various concentrations of PWM. The optimal concentrations for each population was relatively similar, ranging from 0.5 to 2.5 μg PWM/culture, although at lower PWM concentrations, T_G cells responded significantly better than the other T cell subsets. As with the

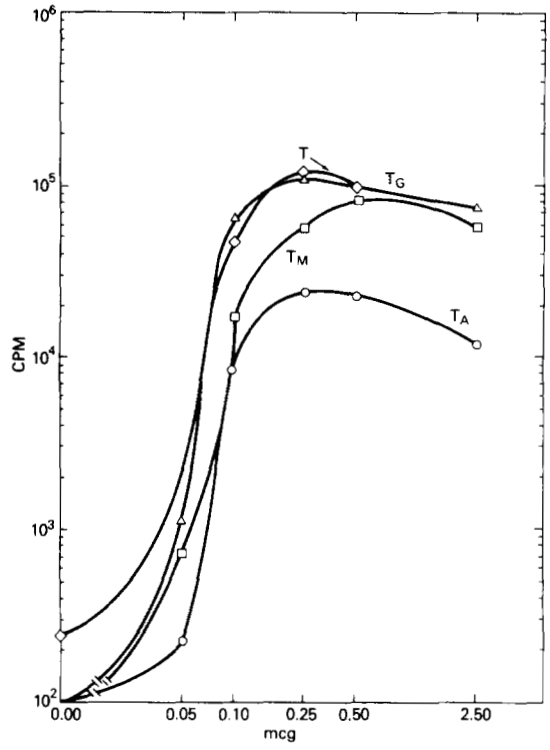

Figure 1. Response of the various T cell subpopulations to PHA after 3 days of culture to various doses of PHA. Each point represents the mean cpm of triplicate cultures.

TABLE I

Phytohemagglutinin-induced proliferation in the various T cell subpopulations (tritiated thymidine incorporation at 3 days)

Expt. No.	T_A	T_G	T_M
1	6,515[a]	38,708	35,621
2	2,119	65,964	39,942
3	2,681	62,291	
4	22,981	82,006	85,883
5	59,534	52,458	63,452
Mean	8,726	58,481	52,765

[a] All values have been corrected for unstimulated background and are reported as mean cpm of triplicate cultures. Background unstimulated values were less than 100 cpm for T_A, T_G, and T_M subpopulations except for experiment 3 in which the T_G unstimulated had a background of 4200 cpm. The dose of PHA in these experiments was 0.5 μg of PHA in 0.20 ml of culture.

TABLE II

Time course of PHA-induced proliferation in T cell subsets

	3 Days	4 Days	5 Days
T	39,687[a]	36,315	22,439
T_A	4,752	7,701	21,431
T_G	15,678	8,628	11,918
T_M	32,920	28,096	28,153

[a] All values in cpm are means of triplicate cultures corrected for background unstimulated cultures. Backgrounds were all less than 300 cpm.

response to PHA, the T_A subset responded much less well than the other T cell populations to PWM. Table III shows three experiments confirming this hyporesponsiveness of the T_A cells to PWM. Time course experiments for the T cell subsets (Table IV) demonstrate that the peak proliferative response of the T_A subset to PWM was delayed in comparison with the other T cell subsets, similar to the shifted kinetics observed in the PHA response.

Role of accessory cells. The positive selection procedure used to purify the T cells in these experiments resulted in a very significant depletion of monocytes from the responding cell populations. To determine if adherent accessory cells might have an influence on the proliferative response of these T cell subsets, irradiated plastic adherent cells were added to cultures of each T cell population, and the response to PHA and PWM was measured. The addition of adherent accessory cells had little or no effect on the response of unfractionated T cells, T_G cells, or T_M cells to PHA. By contrast, adherent accessory cells augmented the response of the T_A subset significantly, 3-fold in the experiment shown in Table V. The response of T cell subpopulations to PWM was enhanced to a variable extent by the addition of irradiated adherent accessory cells. The response of the T_A subpopulation was enhanced to the greatest extent, as in the experiment shown in Table VI, where the response was increased 5-fold.

Mixed lymphocyte culture response of the T cell subpopulation. Table VII shows the results of five experiments com-

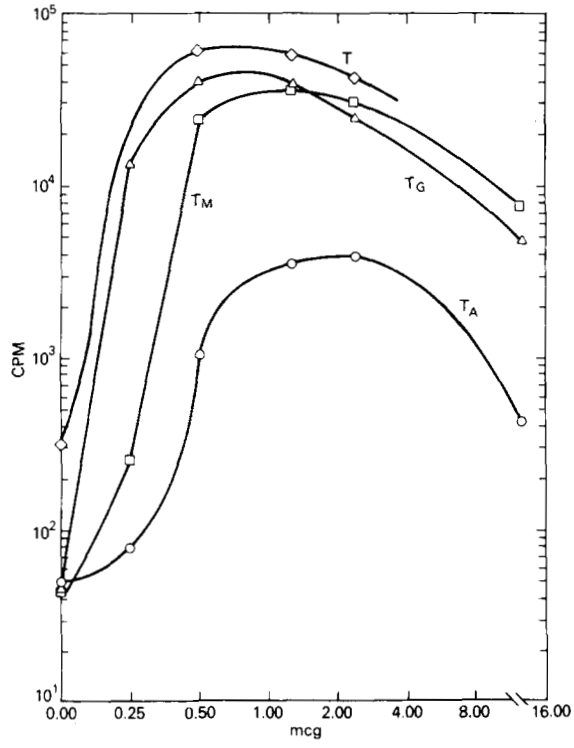

Figure 2. Response of the various T cell subpopulation to PWM after 6 days of culture to various doses of PWM. Each point represents the mean cpm of triplicate cultures.

TABLE III

Pokeweed mitogen-induced proliferation in the various T cell subpopulations (6 days)

Expt. No.	T_A	T_G	T_M
1	1,593[a]	31,482	37,799
2	1,340	31,147	39,747
3	1,399	24,838	30,763
Mean	1,440	28,986	35,887

[a] All values have been corrected for background and are reported as mean cpm of triplicate cultures. Background unstimulated values were less than 100 cpm. The dose of PWM used in these experiments was 2.5 μg.

TABLE IV

Time course of PWM-induced proliferation of T cell subsets[a]

	5 Days	7 Days	9 Days
T	45,595	61,221	27,619
T_A	17,393	73,736	72,879
T_G	35,689	63,807	26,485
T_M	49,279	52,206	27,263

[a] All values are means of triplicate cultures corrected for background unstimulated cultures. Backgrounds were all less than 90 cpm.

TABLE V

The PHA proliferative response in the presence of adherent accessory cells (3 days)[a]

	No Mitogen		0.5 μg of PHA	
	T cells alone	Mϕ added	T cells alone	Mϕ added
T	566	975	69,914	62,316
T_A	105	312	9,398	31,323
T_G	664	572	29,459	33,355
T_M	155	4,308	112,844	102,006

[a] Mϕ indicates the response in the presence of 3×10^4 adherent accessory cells. Values are reported as means of triplicate cultures.

TABLE VI

The PWM proliferative response in the presence of adherent accessory cells (6 days)[a]

	No Mitogen		2.5 μg PWM	
	T cells alone	Mϕ added	T cells alone	Mϕ added
T	4,704	6,451	46,351	66,774
T_A	120	1,865	6,908	34,501
T_G	318	1,731	21,130	47,697
T_M	265	3,338	59,482	140,652

[a] Mϕ indicates the response in the presence of 3×10^4 adherent accessory cells. Values are reported as means of triplicate cultures.

TABLE VII

Response of the various T cell subpopulations in allogeneic mixed lymphocyte cultures (6-day tritiated thymidine incorporation)[a]

Expt. No.	$T_A \times$ Allo*	$T_G \times$ Allo*	$T_M \times$ Allo*
1	19,787	32,984	115,303
2	42,599	51,167	72,713
3	6,662	61,807	66,628
4	53,212	110,552	57,292
5	33,788	20,254	69,082
Mean	25,167	47,171	73,945

[a] Each value was obtained from the mean of triplicate determinations and is reported as counts per minute (cpm). Responder cells (0.1×10^6 of the T cell subsets) and allogeneic mononuclear cells (allo) (0.1×10^6 cells) irradiated with 3000 R (*) as stimulator cells were added to each experiment. $T_A \times T_A$*, $T_G \times T_G$*, and $T_M \times T_M$* backgrounds were always less than 70 cpm.

paring the relative responses of the T cell subpopulation with allogeneic stimulator cells in the mixed lymphocyte culture. The T_M subpopulation generally responded most actively in mixed lymphocyte cultures, the T_G subpopulation was next in activity, and again the T_A subset demonstrated the weakest proliferative response of the various T cell subsets tested.

Helper activity of the T cell subsets for immunoglobulin production. The capacity of the T cell subpopulations to provide helper activity for immunoglobulin production by B cells in cultures stimulated with PWM was determined. Tables VIII and IX show two representative experiments in which varying numbers of unirradiated T cells were added to 2×10^5 B cells in cultures stimulated with PWM. After 6 days of culture, the number of immunoglobulin-secreting cells in these cultures was determined in a RHPA by using an anti-human immunoglobulin (polyvalent) developing reagent (18). In general, T_M cells provided the highest helper activity as revealed by responses at the lowest T to B cell ratios. In these experiments, T_G cells were also capable of providing significant helper activity, although usually considerably less than that provided by T_M cells. As with the other functional properties tested in this study, the T_A subpopulation was capable of providing helper activity for B cell activation, but the magnitude of this function was also lower than that provided by the other T cell subsets.

To determine whether the T_A subset might be restricted in providing helper activity for only a single class of immunoglobulin, similar cultures were set up and the classes of immunoglobulin produced with each T cell subset were determined. Table X shows that helper activity for the production of IgA, IgG, and IgM was present in each of the T cell subsets. Although T_G cells provided little helper activity for IgA production in this experiment, T_G cells provided excellent help for IgA production in other experiments. In none of the experiments shown with normal donors was excessive suppressor T cell activity found in any of the T cell subpopulations.

DISCUSSION

The recognition that human T cell subsets can be identified and isolated on the basis of unique cell surface Fc-receptors has

TABLE VIII

Plaque-forming response of the various T cell subpopulations in PWM-induced immunoglobulin production (total polyvalent plaque-forming cells/culture)[a]

T:B Ratios	T_A	T_G	T_M
0.015	1	2	2
0.15	6	93	719
0.25	313	2,893	2,203
1.5	1,410	11,029	8,830
5.0	1,550	3,174	5,757

[a] B cells alone or B cells in the presence of PWM always made <5 PFC/culture. T cells or the T cell subpopulations made <5 PFC/culture.

TABLE IX

Plaque-forming response of T cell subpopulations in PWM-induced immunoglobulin production (PFC/culture at 7 days)[a]

T:B Ratio	T_A	T_G	T_M
1.0	882	227	4585
2.5	633	970	2046
4.0	243	1208	4439

[a] B cells alone in the presence of PWM made <5 PFC/culture. T cells in the presence of PWM made <5 PFC/culture. PFC were developed with a polyvalent anti-human immunoglobulin reagent in the reverse hemolytic plaque assay.

TABLE X

Class of immunoglobulin produced in the presence of the various T cell subpopulations[a]

	IgA PFC	IgG PFC	IgM PFC
0.3 B + 0.9 T	191	911	799
0.3 B + 0.9 T_A	108	161	359
0.3 B + 0.9 T_G	12	167	258
0.3 B + 0.9 T_M	247	796	1096

[a] T, T_A, T_G, and T_M alone produced <5 PFC/culture in the presence of PWM. B cells alone in the presence of PWM made <5 PFC/culture.

spurred investigation of these cellular subsets in the hope that they will prove to have distinctly different functional properties. The observation that the Ly system of alloantigens on murine T cells can be used to identify subsets of T cells with different functions has strengthened this prospect (20). Studies of patients with immunodeficiency disease, lymphoid malignancy, and others, have clearly demonstrated that functionally distinct T cell subsets do exist in man (21, 22).

The initial exciting reports of the functions of human T_M and T_G cells suggested that these markers might, in fact, identify T cells with very different functional properties. Moretta *et al.* (10) showed that T_G and T_M cells had different dose-response profiles in response to stimulation with PHA. Even more provocative were the observations that T_M cells provided helper activity for PWM-induced B cell activation, whereas T_G cells did not (11). In fact, the T_G subset was found to actively suppress the PWM response seen in cultures containing B cells and T_M cells (11). The suppressor activity of T_G cells appeared to require activation of these cells by both PWM and IgG antibody-antigen complexes (12). Further evidence for functional differences between T_M and T_G cells was obtained in studies of lectin-induced cellular cytotoxicity. T_M and T_G cells were found to express different spectra of cytotoxic activity toward various target cells in the presence of PHA (15).

Recent evidence, however, has shown that the distinction between the functions of T_M and T_G cells may not be as clear as originally thought. T_M cells have been demonstrated to contain suppressor activity in the human newborn and in adult T cells after *in vitro* stimulation with concanavalin A (13, 23). Furthermore, the Fc-receptor markers are not stable. Pichler *et al.* (24) demonstrated that T_G cells, after interaction with IgG antibody-antigen complexes, lose their Fc-IgG receptors and then express Fc-IgM receptors. Thus, T_G cells may become T_M cells after IgG immune complex interaction. By contrast, the Ly alloantigens on murine T cells are thought to be stable, in that Ly 1-bearing cells do not change to express Ly 2,3, and vice versa. Ly 1,2,3 cells may, however, differentiate to express either Ly 1 or Ly 2,3 antigens (20).

In this report, we have described the functional properties of the subpopulation of human T cells that bear Fc-IgA receptors. These T cells appear to be relatively distinct from the T_M and T_G subsets in several respects. They are generally much weaker responders in all of the *in vitro* assays studied. They also appear to be more dependent upon accessory cells for proliferation to mitogens than are the other subsets. They also respond to PHA and PWM with a delayed peak response compared with T_M and T_G cells.

Since this subset of cells is prepared by using essentially identical methodology to that used to isolate the other T cell subsets, it is unlikely that these results can be explained by a technical artifact such as selective cell damage acquired during isolation. If this were the case, then this T cell subset is unique in its susceptibility to damage. Furthermore, cell viability as assessed by trypan blue exclusion was essentially identical for all T cell subsets. The possibility that the responses observed in the T_A subset are really due to contaminating T_M or T_G cells is difficult to rule out completely. However, the T_A cells formed very tight rosettes, and on the average, 95% of the cells in the T_A pellet were rosetted with three or more ORBC. Furthermore, the relative differences between function among the T cell subsets, such as accessory cell dependence, different kinetics, and relatively more preserved mixed lymphocyte culture reactivity compared with PWM responsiveness among the T_A cells, make this possibility less likely. Even if all reactivity in the T_A subset was due to the small contamination with other T cell subsets, then the T_A population would be even more distinctive for its lack of intrinsic reactivity.

The results of these studies differ from previously published reports in that we found T_G cells to consistently provide helper activity for B cell activation in PWM-driven cultures, whereas Moretta *et al.* (11, 12) not only failed to see help with this subset, but actually observed suppression in the presence of help provided by T_M cells. In over 15 experiments with highly purified T cell subsets isolated from normal donors, we have observed suppression by the T_G subset in less than one-third. In the remainder of these experiments, the T_G cells provided help as they did in all the experiments comparing T_G, T_M, and T_A subsets. Although the degree of help provided by the T_G cells usually was considerable, it rarely was as great as the helper activity provided by T_M cells. The reason for this discrepancy between our results and those of Moretta *et al.* are not clear. Perhaps the different preparations of PWM or FCS used in the two studies may account for the differences, or slight differences in the technique or reagents used to isolate the T cell subsets may account for the varying results.

The actual role that the Fc receptors play in T cell function is still largely not understood. Our data that T cells from each subset can provide helper activity for all three classes of immunoglobulin demonstrate that the Fc receptor-bearing T cells are not restricted to interacting with only a single class of potential immunoglobulin-producing cells. The possibility that feedback by antigen-antibody complexes might modulate the function of certain Fc receptor-bearing T cell subsets to become active suppressor cells has been suggested (11, 12, 25), but the T cells in our studies were also positively selected by antibody-antigen complex interaction, and we did not observe suppressor activity. These observations suggest that there are quantitative but not necessarily qualitative differences in the functional properties of the human T cell subsets characterized by Fc receptors for IgA, IgG, and IgM, and that there is not a strict analogy between this system of cell surface receptors and the murine Ly alloantigen system.

Acknowledgments. The authors wish to gratefully acknowledge the expertise of Mrs. Nancy Dooley and Mrs. Irma Koski for their technical assistance.

REFERENCES

1. 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.
2. 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.
3. Gmeling-Meyling, F., M. Van der Ham, and R. E. Ballieux. 1976. Binding of IgM by human T lymphocytes. Scand. J. Immunol. 5: 487.
4. McConnell, I., and C. M. Hurd. 1976. Lymphocyte receptors. II. Receptors for rabbit IgM on human T lymphocytes. Immunology 30:835.
5. 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.
6. Moretta, L., M. C. Mingari, S. R. Webb, E. R. Pearl, P. M. Lydyard, C. E. Grossi, A. R. Lawton, and M. D. Cooper. 1977. Imbalance in T-cell subpopulations associated with immunodeficiency and autoimmune syndromes. Eur. J. Immunol. 7:696.
7. Gupta, S., and R. A. Good. 1978. Subpopulations of human T lymphocytes V. T lymphocytes with receptors for immunoglobulin

M or G in patients with primary immunodeficiency disorders. Clin. Immunol. Immunopathol. 11:292.
8. Trompteter, R. S., Layward, L., and A. R. Hayward. 1978. Primary and secondary abnormalities of T cell subpopulations. Clin. Exp. Immunol. 34:388.
9. Gupta, S., and R. A. Good. 1978. Subpopulations of human T lymphocytes. III. Distribution and quantitation in peripheral blood, cord blood, tonsils, bone marrow, thymus, lymph nodes, and spleen. Cell Immunol. 36:263.
10. 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.
11. Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydyard, and M. D. Cooper. 1977. Functional analysis of two human T-cell subpopulations: receptors for IgM (T_M) or IgG (T_G). J. Exp. Med. 146:184.
12. Moretta, L., M. C. Mingari, A. Moretta, and M. D. Cooper. 1979. Human T lymphocyte subpopulations: studies of the mechanism by which T cells bearing Fc receptors for IgG suppress T-dependent B cell differentiation induced by pokeweed mitogen. J. Immunol. 122:984.
13. 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.
14. Pichler, W. J., F. W. Gendelman, and D. L. Nelson. 1979. Fc-receptors on human T lymphocytes. II. Cytotoxic capabilities of human T_G, T_M, B, and L cells. Cell. Immunol. 42:410.
15. Lum, L. G., A. V. Muchmore, J. M. Decker, and R. M. Blaese. 1979. MICC cytotoxic effector function of human T lymphocyte subpopulations bearing Fc-receptors for IgG and IgM. Clin. Exp. Immunol. 37:558.
16. Lum, L. G., A. V. Muchmore, D. Keren, J. Decker, I. Koski, W. Strober, and Blaese, R. M. (1979) A receptor for IgA on human T lymphocytes. J. Immunol. 122:65.
17. Pellegrino, M. A., S. Ferrone, M. P. Dierich, and R. A. Reisfeld. 1975. Enhancement of sheep red blood cell human lymphocyte rosette formation by the sulfhydryl compound 2-aminoethylisothiouronium bromide. Immunol. Immunopathol. 3:324.
18. Koski, I. R., D. C. Poplack, and R. M. Blaese. 1976. A nonspecific esterase stain for the identification of monocytes and macrophages. *In In vitro* cell mediated and tumor immunity. Edited by B. R. Bloom and M. R. David. Academic press, New York. P. 359.
19. Lawrence, E. C., R. M. Blaese, R. R. Martin, and P. M. Stevens. 1978. Immunoglobulin secreting cells in normal human bronchial lavage fluids. J. Clin. Invest. 62:832.
20. Cantor, H., and E. A. Boyse. 1977. Lymphocytes as models for the study of mammalian cellular differentiation. Immunol. Rev. 33:105.
21. Broder, S., E. Lawrence, M. Durm, C. Goldman, L. Muul, and T. A. Waldmann. 1977. Further characterization of neoplastic helper T cells from patients with the Sezary syndrome. *In* Regulatory Mechanisms in Lymphocyte Activation. Edited by D. O. Lucas. Academic Press, New York. P. 689.
22. Broder, S., D. Poplack, J. Whang-Peng, M. Durm, C. Goldman, L. Muul, and T. Waldmann. 1978. Characterization of a suppressor-cell leukemia: evidence for the requirement of an interaction of two T cells in the development of human suppressor effector cells. N. Engl. J. Med. 298:66.
23. 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.
24. Pichler, W. J., L. G. Lum, and S. Broder. 1978. Fc-receptors on human T lymphocytes. I. Transition of $T\gamma$ to $T\mu$ cells. J. Immunol. 121:1540.
25. Bich-Thuy, L. T., 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.