HUMAN MALIGNANT T CELLS CAPABLE OF INDUCING AN IMMUNOGLOBULIN CLASS SWITCH

By LLOYD MAYER, DAVID N. POSNETT, and HENRY G. KUNKEL

From the Department of Immunology, The Rockefeller University, New York 10021

There is considerable controversy regarding the role of T cells in the regulation of immunoglobulin class switching. There is little question that B cells can switch Ig isotype in the absence of T cells (1–3), and that these events can occur randomly (4) or according to gene order (5, 6). However, evidence is accumulating to suggest that T cells play an important role in this event. Immunization with various T-dependent antigens results in selective Ig class expression (7–9) and even isotype responses to thymus-independent antigens can be influenced by T cells (5, 6, 10–12). However, T cell regulatory influences in these cases may relate to a nonswitching event, i.e., expansion of cells precommitted to isotype. Such examples of T cell expansion of isotype-committed B cells are well documented in both antigen-specific (13) and nonspecific systems (14).

Direct evidence for T cell regulation of Ig class switching is not as well documented. Two studies, one by Isakson et al. (15) and one by Kawanishi et al. (16), provide the best evidence to date regarding the existence of "switch" T cells. Isakson et al. (15) demonstrated a B cell differentiation factor (BCDF)γ^1 derived from a murine T cell clone that induces an increase in γ_1 secretion from lipopolysaccharide and BCDF-treated μ^+ B cells. Kawanishi and co-workers (17) described a T cell clone derived from Peyer's patches that appears to direct surface IgM (sIgM)-bearing B cells to become sIgA$^+$. In the latter case, no IgA secretion is noted unless a nonspecific terminal differentiation signal is added (17). In both of these cases, however, it is unclear whether the target populations have not already undergone a recombinational event in the Ig heavy chain gene, committing them to a given isotype. If this were the case, then the T cells described above would only be expanding an isotype-precommitted B cell population. This problem is difficult to resolve in view of the fact that heterogeneous B cell populations are used in these studies and the status of the constant region heavy chain (C_H) gene has not been evaluated.

In the present study, the malignant T cells derived from a patient with a mycosis fungoides/Sezary's syndrome were found to promote nonspecific Ig class switching from IgM to IgG and IgA. This was evident in several assay systems.

This work was supported by grants AI-19080 and AI-10811 from the U.S. Public Health Service.
Address correspondence to L. Mayer at (current address) Mount Sinai Hospital, Annenberg 23-08, 1 Gustave L. Levy Place, New York, NY 10029.

[1] *Abbreviations used in this paper:* BCDF, B cell differentiation factor; BCGF, B cell growth factor; C_H, constant region heavy chain; CM, complete culture medium; MNC, mononuclear cells; ORBC, ox red blood cells; PBS, phosphate-buffered saline; PFC, plaque-forming cell; PWM, pokeweed mitogen; sIg, surface immunoglobulin.

In addition, and as further evidence that these malignant cells were switch T cells, the switching defect in a patient with immunodeficiency and hyper-IgM was corrected in vitro.

Materials and Methods

Patient Material. Heparinized blood was obtained from patient Rac, a patient with a mycosis fungoides/Sezary-like syndrome characterized by the presence of papular skin lesions with Sezary cells noted on biopsy. There were no classical Pautrier's abscesses present. The patient's serum Ig profile consisted of an IgG of 2,480 mg%, an IgA of 1,140 mg%, and no detectable IgM, as determined by standard radial immunodiffusion technique (18). A bone marrow aspirate revealed 13% plasma cells. Of these, ~50% contained IgG and 50% contained IgA. Very few IgM plasma cells were found. Peripheral blood smears revealed 10–30% large irregular lymphoid cells with lobulated nuclei, consistent with Sezary cells. Results of immunofluorescent staining of cell surface markers on the patient's mononuclear cells (MNC) are listed in Table I.

HIV is a patient with acquired immunodeficiency and hyper-IgM as described by Cruchaud et al. (19). The serum Ig profile consisted of absent IgG and IgA, with an IgM of 1,280 mg%.

Cell Separation and Culture Conditions. 50 ml of heparinized venous blood were obtained from normal laboratory volunteers as well as the patients described above, diluted 1:3 with sterile phosphate-buffered saline (PBS), layered on a Ficoll-Hypaque density gradient (Pharmacia Fine Chemicals, Piscataway, NJ), and centrifuged for 30 min at 500 *g*. The interface cells (MNC) were washed three times in PBS before use. Tonsillar MNC were obtained from routine tonsillectomy specimens, as previously described (14). T and B cell separation was achieved by a rosetting technique using neuraminidase-treated sheep red blood cells (SRBC) and Ficoll-Hypaque density gradient centrifugation (14).

All cells were cultured in RPMI 1640 (M. A. Bioproducts, Walkersville, MD), 10% fetal calf serum (Reheis Co., Inc., Phoenix, AZ), 1% penicillin, streptomycin (Gibco Laboratories, Grand Island, NY), and 2 mM glutamine (Gibco Laboratories), hereafter designated as complete culture medium (CM). In certain experiments, pokeweed mitogen (PWM) (1:100 dilution; Gibco Laboratories) was added.

Isolation of sIgM$^+$ Tonsillar B Cells. Tonsillar MNC were separated into T and non-T fractions as described above. Interface cells were subjected to a second rosetting to remove contaminating T cells (resultant populations <0.5% OKT3$^+$). Non-T cells were washed three times in PBS and diluted in 5 ml of CM at a concentration of 4×10^6/ml. Ox RBC (ORBC) were coated with an F(ab')$_2$ affinity-purified rabbit anti-human IgG or IgA, using a chromium chloride technique (20). One ml of a 5% solution of the coated ORBC was added to the tonsilar non-T cells, centrifuged at 200 *g* for 5 min, and incubated for 20 min at 4°C. The pellet was resuspended in CM, layered on a Ficoll-Hypaque gradient, and centrifuged at 500 *g* for 30 min at 4°C. The interface cells were sIgM$^+$ with rare contaminating sIgG$^+$ and sIgA$^+$ cells. Immunofluorescent staining of these interface cells with previously described fluorescein-conjugated, affinity-purified F(ab')$_2$ reagents revealed the following: sIgM, 99%; sIgG, 0.3%; and sIgA, 0.01%.

Isolation of Tac$^+$ and Tac$^-$ cells. The monoclonal antibody Tac (anti-TCGF [T cell growth factor] receptor) was kindly provided by Dr. Thomas Waldmann. To isolate the Tac$^+$ cells from patient Rac (30% of peripheral blood lymphocytes [PBL]), an indirect rosetting technique was performed using F(ab')$_2$ affinity-purified goat anti-mouse Ig–coated ORBC (14). Resting T cells from normal controls were uniformly Tac$^-$ and, therefore, no isolation was performed on these populations. 40 million T_{Rac} cells were incubated with Tac antibody (1:40,000 dilution of original ascites in CM) for 30 min at 22°C. Cells were washed three times in CM and resuspended in 0.5 ml CM. 0.5 ml of a 2% Ig-coated ORBC solution was added and the mixture was kept on ice for 5 min. The cells were then spun at 200 *g* for 5 min at 4°C and further incubated on ice for 30 min. The pellet was gently resuspended and layered on a Ficoll-Hypaque density gradient. The

resultant pellet (Tac^+ cells) was subjected to hypotonic lysis with 0.84% ammonium chloride for 5 min on ice. The interface cells were Tac^- (<1% staining with Tac).

Immunofluorescence. Standard direct and indirect immunofluorescence was performed as previously described (21). A cytofluorograph (30-H; Ortho Diagnostic Systems, Inc., Westwood, MA) was used for all fluorescence analysis. OKT3, OKT4, OKT8, and OKM1 were obtained from Ortho Diagnostic, Raritan, NJ.

Plaque Assay. A reverse hemolytic assay using protein A–coated SRBC was performed as previously described (22). Affinity-purified rabbit anti-human IgG, IgA, and IgM were obtained from Cappel Laboratories, Cochranville, PA and used at a 1:100 dilution as developing antisera. All cultures were assayed on day 6 unless otherwise indicated. In some experiments, T cells were irradiated (1,500 rad) using a cesium source (Isomedix, Inc., Whippany, NJ), before addition to culture.

Results

Characteristics of MNC, T Cells and B Cells from Patient Rac. The peripheral blood of this patient contained a malignant clone of T cells that accounted for approximately one-third of the MNC. This was especially evident from the blastoid appearance of these cells and the presence of the Tac antigen on their membrane (Table I). This picture was similar to that often observed in the type of Sezary cell disease with primary cutaneous manifestations of mycosis fungoides. The Tac positivity permitted isolation of these cells from the peripheral blood. Table I summarizes the results of cell surface staining using various monoclonal antibodies and $F(ab')_2$ affinity-purified rabbit anti-human IgG, IgA, IgM, and IgD. Other than the Tac positivity noted above, results were unremarkable.

Ig analysis of the serum of this patient showed a selective depletion of IgM. Experiments were therefore initiated to determine where the defect in IgM production existed. As seen in Table II (one representative experiment in a series of four), no IgM plaque-forming cells (PFC) were noted when MNC-Rac were cultured in the presence of PWM, whereas IgG and IgA PFC, although consistently lower than the normal control, were present. Co-culture of non-T

TABLE I
Cell Surface Staining

	Rac-MNC	T_{rac}*	HIV-MNC
	%	%	%
OKT3‡	66.0	98.3	68.0
OKT4	42.0	—§	39.5
OKT8	19.4	—	22.4
κ/λ	6.9	0.4	8.6
OKM1	12.9	2.6	10.2
$sIgG^+$	2.0	—	<0.5
$sIgA^+$	0.9	—	<0.5
$sIgM^+$	0.4	—	3.6
$sIgD^+$	0.5	—	—
Tac	—	30.2	—
Ia	—	24.6 (weak)	—

* Isolated T cells from Rac-MNC.

‡ 2×10^5 peripheral blood MNC or T cells from patient Rac or HIV were stained by direct or indirect immunofluorescence (see Materials and Methods). Results are expressed as percent positivity.

§ Not done.

TABLE II
Characteristics of Unseparated MNC, T Cells, and B Cells from Patient Rac

Target	Added cells	PWM	PFC/well*		
			G	A	M
MNC Rac	–	–	0	80	0
Rac	–	+	740	600	0
Control	–	–	50	20	90
Control	–	+	12,090	880	6,760
Non-T Rac	–	+	0	40	0
	T_{rac}	+	2,460	560	0
	$T_{control}$	+	3,130	860	0
Non-T control	–	+	220	90	120
	T_{rac}	+	5,010	1,420	10
	$T_{control}$	+	15,000	510	2,190

* One million MNC were cultured with or without PWM or 1×10^6 non-T cells were co-cultured with or without allogeneic T cells (1×10^6) in the presence of PWM. A reverse hemolytic plaque assay was performed on day 6, using class-specific developing antisera.

cells from patient Rac with either autologous or allogeneic control T cells and PWM yielded similar results, with no IgM PFC but good IgG and IgA responses. The addition of Epstein-Barr virus (EBV) to Rac non-T cells however, did result in the production of some IgM PFC, indicating that the cellular machinery necessary for IgM production and secretion was still present in some cells (data not shown).

Experiments were performed to determine whether the T cells from Rac (T_{rac}) were playing a role in the diminished IgM production. Co-culture of control non-T cells with T_{rac} and PWM resulted in a marked absence of IgM PFC (Table II), whereas IgA production was unchanged and IgG was only slightly decreased, or unchanged, when compared with control autologous T cells. To determine whether this finding was due to IgM class-specific suppression, two assay systems were used. In the first, 1×10^6 allogenic MNC and PWM were cultured with 1×10^6 T_{rac} or normal allogeneic T cells. No suppression was noted of any Ig class, either with irradiated or unirradiated T_{rac} or two allogeneic control T cell populations (Table III). In the second system, irradiated or unirradiated T_{rac} or control T cells were added to three different lymphoblastoid B cells lines, SeD (IgM, κ), 8866P (IgG, κ), and 32a1 (IgA, λ) and tested for suppression of Ig secretion. 1×10^5 T_{rac} or control T cells were added to 1×10^4 or 1×10^5 lymphoblastoid cells, and class-specific Ig secretion was measured at 12, 24, 48, 72, and 96 h. No suppression was seen with T_{rac} or control T cells when added to either 8866P or 32A1 (data not shown). Suppression (30–50%) was noted, however, when T cells were co-cultured with SeD, but this response was not specific for T_{rac}. In fact, T_{rac} exhibited the least suppression of the three T cell populations used, at any time tested (data not shown). Thus, since the mechanism of reduced IgM secretion does not appear to be mediated through class-specific

TABLE III
Lack of IgM Suppression by T_{rac} Cells

Target	Allogeneic cells	PFC/well*		
		G	A	M
MNC control	—	9,260	8,110	4,200
	T_a	12,560	7,650	4,490
	T_b	8,770	5,490	3,550
	T_{rac}	11,680	6,820	3,860
	T_a-X‡	10,660	4,980	6,090
	T_b-X	9,880	4,600	5,600
	T_{rac}-X	8,600	2,120	2,900

* As per Table II. One million MNC were co-cultured with 1×10^6 allogeneic T cells in the presence of PWM (1:100 dilution).
‡ Irradiated (1,500 rad).

suppression, experiments were performed to determine whether T_{rac} was inducing a class switch.

Ability of T_{rac} to Induce a Class Switch from IgM to IgG and IgA. A model for the assay of T cell regulation of Ig class switching was established as follows. $sIgM^+$ tonsillar B cells were used (see Materials and Methods). These were depleted of significant sIgG and sIgA by a direct rosetting technique. IgG and IgA produced after stimulation with T cells was considered evidence suggestive of a class switch, since cells definitively committed to other isotypes were already depleted.

One million μ^+ tonsillar B cells were co-cultured with 1×10^6 T_{rac} or one of two different allogeneic T cell controls and PWM. In three separate experiments (Table IV), the addition of allogeneic T cells to the μ^+ tonsillar B cells resulted in the production of increased IgM PFC (range, 5,620–9,870). In addition, IgG and IgA production was also noted (range, 1,760–7,690 and 880–6,400, respectively), although generally lower than that seen with unfractionated B cells (Table IV legend and data not shown). However, addition of T_{rac} cells to the μ^+ tonsillar B cells resulted in little or no IgM PFC (range, 0–220) but induction of IgG and IgA PFC (range, 650–1,000 and 220–2,000, respectively) was still noted. The number of IgG and IgA PFC never achieved the levels seen with normal control T cells but they were always significantly higher than the number of IgM PFC.

Evidence that the Tac^+ T_{rac} Cells Are Important in the Switching Process. T_{rac} cells were depleted of the 30% Tac^+ cells by indirect rosetting technique (see Materials and Methods). The resulting Tac^- population was no longer capable of inducing an Ig class switch (Table V; one representative experiment of three with non-T cells) (5,580 vs. 40 PFC and 290 vs. 0 PFC for IgG and IgA, respectively). In contrast, the directly isolated Tac^+ cells did induce IgG and IgA PFC (5,580 vs. 2,080 PFC and 290 vs. 190 PFC for IgG and IgA, respectively).

Unfractionated T_{rac} cells gave rise to higher numbers of IgG and IgA PFC, suggesting the necessity for additional helper T cells in the differentiation process. The addition of Tac antibody to the cultures (1:40,000 dilution at the concentration used for rosetting) (Table V) or a range of concentrations (1:10,000 to 1:400,000) (data not shown) had no effect on B cell differentiation

TABLE IV

T_{rac} Induces an Ig Class Switch in μ^+ Tonsillar B Cells

Exp.	Added cells	PFC/well*		
		G	A	M
1‡	—	0	0	10
	T_a	5,000	6,400	8,000
	T_b	7,690	3,400	5,900
	T_{rac}	1,000	2,000	220
2	—	0	0	40
	T_c	4,290	2,860	9,870
	T_d	2,650	1,760	5,620
	T_{rac}	880	420	0
3	—	0	0	90
	T_b	6,730	1,340	8,320
	T_c	1,760	880	7,810
	T_{rac}	650	220	10

* As per Table II. All cultures performed in the presence of PWM.

‡ μ^+ tonsillar B cells were selected as described in Materials and Methods. In experiment 1, co-culture of T_a with unseparated tonsil B cells in the presence of PWM resulted in 12,860, 9,870, and 12,990 PFC for IgG, IgA, and IgM, respectively. T_b and tonsil B cells resulted in 16,980, 7,630, and 12,230 PFC for G, A, and M, respectively. T_{rac} and tonsil B cells resulted in 2,340, 1,890, and 10 PFC for G, A, and M, respectively. Results in the other experiments were similar.

TABLE V

Role of Tac^+ T Cells in the Switching Process

Target cells	Added cells	PFC/well*		
		G	A	M
Peripheral blood non-T	—	70	20	110
	$T_{autologous}$	8,870	980	5,460
	T_{rac}	5,580	290	90
	T_{rac} minus Tac^+ cells‡	40	0	20
	Tac^+ T cells	2,080	190	30
	Tac antibody (1:40,000)	30	0	10

* As per Table II. All cultures performed in the presence of PWM.

‡ Tac^+ cells were isolated by an indirect rosetting technique as described in Materials and Methods.

or isotype expression. Since there are no resting Tac^+ T cells in normal MNC, control depletion of Tac^+ cells could not be performed.

T_{rac} Induces a Class Switch in Cells from a Patient With Immunodeficiency and Hyper-IgM. Since μ^+ tonsilar B cells may already be committed to other isotypes at the DNA level but not yet express sIgG or sIgA, it was necessary to find a better model system for Ig class switching. Immunodeficiency with hyper-IgM is a syndrome characterized by normal to elevated levels of IgM with undetectable IgG and IgA (19, 23, 24). Previous attempts (25–26) to induce IgG and IgA secretion in cells from these patients in vitro have been unsuccessful using various B cell mitogens and allogeneic T cells. Although certain groups (26) claim that

TABLE VI

T_{rac} Induces an Ig Class Switch in Cells from a Patient (HIV) with the Hyper-IgM Syndrome

Target cells	Added cells	PFC/well*		
		G	A	M
HIV MNC	—	0	0	5,690
	$T_{control}$	0	0	8,750
	T_{rac}	550	0	0
HIV non-T	—	0	0	110
	$T_{control}$	0	0	15,980
	T_{rac}	230	0	10
	$T_{autologous}$	0	0	9,770

* As per Table II. All cultures performed in the presence of PWM. T_{rac} or $T_{control}$ cells in the presence of PWM did not secrete any Ig in several experiments.

this syndrome represents an intrinsic B cell defect, the possibility remains that, in certain patients, there is a defect in switch signals from T cells. To address this possibility, 1×10^6 T_{rac} or other allogeneic T cells were added to either 1×10^6 MNC or non-T cells from patient HIV (Table VI), with immunodeficiency and hyper-IgM. As seen in Table VI, 1×10^6 HIV-MNC stimulated with PWM yielded 5,690 IgM PFC but no IgG or IgA PFC. Addition of allogeneic control T cells resulted in a slight increase in IgM PFC (8,750 PFC/culture) but still no IgG or IgA PFC. However, when T_{rac} cells were added, no IgM PFC and 550 IgG PFC were noted. Similar data were obtained using non-T cells from this patient (Table VI). T_{rac} or allogeneic T cells alone in the presence of PWM yielded no PFC response in numerous experiments (Table VI, legend).

Discussion

We have demonstrated the existence of a T cell (T_{rac}) derived from a patient with a mycosis fungoides/Sezary's syndrome, which is capable of inducing an Ig class switch from IgM to IgG and IgA. The evidence for this comes from two different assay systems. Negatively selected μ^+ tonsillar B cells were used as a model for Ig switching. In the absence of T cells and mitogen, IgG and IgA PFC are not demonstrable (Table IV). However, when T_{rac} cells were added, IgG and IgA PFC were noted, with little or no IgM secretion. Since our data show that IgM production was not actively suppressed by T_{rac} (Table III), these cells appear to be inducing a nonspecific Ig class switch. This does not address the issue that $sIgM^+$ B cells might already be committed to other isotypes at the DNA level. It is still possible that T_{rac} cells might be expanding populations of IgG- and IgA-committed cells. Indeed, control allogeneic T cells were also capable of inducing IgG and IgA PFC in this system, although, in contrast to T_{rac}, significant IgM PFC were also noted (Table IV). Therefore, on the basis of the above data, either switch T cells are present in small numbers in the normal T cell population, with effects amplified by the large numbers of other T cells secreting various B cell growth and differentiation factors, or the μ^+ tonsillar B cell assay only depicts the expansion of isotype-committed sIgM-bearing cells.

This same problem has plagued the other studies (15–17) that described switch T cells or factors and where mitogen-stimulated heterogeneous B cell populations were used. To resolve this issue, a source of B cells without any evidence of Ig gene rearrangement must be used as the target population. To this end we have obtained cells from a patient with immunodeficiency and hyper-IgM. As described by Cruchaud et al. (19), this syndrome is characterized by absent serum IgG and IgA with normal to high levels of serum IgM. In vitro studies (25–26) performed with B cells from these patients confirmed the lack of IgG and IgA secretion, using various stimuli including normal allogeneic T cells. In addition, the genes encoding for the C_H region have been found to be in the germline configuration (S. P. Kwan and F. Rosen, personal communication; in various patients including HIV). Thus B cells from these patients appear to meet the criteria for a model of T cell influence in the regulation of Ig class switching, and should help resolve whether T_{rac} effects a class switch. The data in Table VI suggest that this indeed is the case. Co-culture of normal allogeneic T cells and PWM, or PWM alone, with peripheral blood MNC or B cells derived from a patient with immunodeficiency and hyper-IgM, resulted only in an IgM PFC response (Table VI). These data are consistent with other in vitro studies in these patients (25–26). When T_{rac} cells and PWM are co-cultured with these same B cells, however, IgG PFC are noted, with a concomitant decrease in IgM secretion. These findings are not confined to the one patient (HIV) described here, as we have now screened several other patients with this disorder who are capable of secreting IgG and/or IgA in the presence of T_{rac} cells (Mayer, Kwan, Thompson, Chiorazzi, Rosen, and Kunkel, manuscript in preparation). This suggests that there may be a defect, at least with some patients with this syndrome, in "switch" T cells. The fact that normal T cells are unable to correct this defect, as shown here and as previously documented (26), might reflect quantitative differences in switch signals elaborated by normal and T_{rac} cells. Alternatively, it may reflect the lack of an appropriate activation stimulus in the normal T cell population. T_{rac} cells appear to be a malignant expansion of in vivo activated switch T cells, as evidenced by the finding that the Tac^+ T_{rac} cells appear to be responsible for the switch-inducing activity (Table V) and by the Tac^+ malignant cells on the skin biopsy of patient Rac (data not shown).

The activation signal for the switch T cells is unknown but it might relate to some T-dependent antigens known to enhance non-μ Ig responses (7–9). PWM does not appear to provide an optimal stimulus for Ig class switching (27–28), although some low-level switching might still occur. This might explain, in part, why normal T cells in the presence of PWM are unable to induce an Ig class switch in B cells from patients with the hyper-IgM syndrome. It might also explain why T cells from patients with this syndrome function normally in B cell differentiation assays, (26). Although these patients may have a defect in switch T cells, other T cell functions, such as secretion of B cell growth and differentiation factors, are presumably intact. Thus, co-culture of hyper-IgM T cells with IgG- and IgA-committed B cells would result in IgG and IgA secretion, respectively.

The mechanism by which T_{rac} cells induce an Ig class switch is unclear. It is possible that the T cell secretes a recombinase or recombinase activator (29).

However, supernatants from lectin-stimulated T_{rac} cells or from a Tac^+, IL-2-dependent T_{rac} cloned cell line (active in inducing an Ig class switch) have failed to induce switching. These supernatants are also deficient in nonspecific BCDF or BCGF, which may account for the relatively low levels of IgG and IgA PFC seen in our experiments. Thus, the presence of T_{rac} cells appears to be necessary for actual switching to occur, although we cannot rule out the possibility that switch factors are present in concentrations too low to have any effect in our assay systems. The presence of both IgG and IgA PFC suggests that the T_{rac} signal might mediate an early, nonspecific event, shutting off μ or δ secretion, and allowing for other switch signals to act more specifically. This is supported by the finding that sIgD is rapidly lost from the B cell surface within 12 h of co-culture with T_{rac} (Mayer, Kwan et al., manuscript in preparation).

The accumulated evidence suggests that there is a T cell capable of regulating Ig class switching. Whether this malignant clone of T cells is a part of the normal physiologic process (part of a series of switch T cells) is currently being investigated. The finding of a reversal of a switching defect in patients with immunodeficiency and hyper-IgM suggests that this may be so.

Summary

Evidence is presented for the existence of a "switch" T cell derived from a patient with mycosis fungoides/Sezary's syndrome. The serum immunoglobulin profile in this patient revealed high IgG and IgA but no detectable IgM. Peripheral blood mononuclear cells from this patient secreted only IgG and IgA in the presence of pokeweed mitogen. T cells (T_{rac}) co-cultured with normal allogeneic non-T cells and pokeweed mitogen resulted in only IgG and IgA PFC, with little or no IgM secretion. There was no evidence of active suppression of IgM. Rather, these T cells appeared to induce an Ig class switch from IgM to IgG and IgA, when co-cultured with μ^+ tonsillar B cells. Further evidence was obtained using mononuclear cells derived from a patient with immunodeficiency and hyper-IgM, a syndrome characterized by a lack of IgG and IgA secretion. The addition of T_{rac} cells to either peripheral blood mononuclear cells or non-T cells from a patient with hyper-IgM syndrome resulted in new secretion of IgG, with a concomitant decrease in IgM secretion, whereas control T cells were not effective in inducing secretion of any isotype other than IgM. Isolated Tac^+ T cells from T_{rac} appear to be responsible for this effect.

We wish to thank Christopher Thompson for his invaluable technical assistance, Pamela Bolton for help in preparing this manuscript, and Dr. Nicholas Chiorazzi for supplying cells from patient HIV.

Received for publication 6 September 1984.

References

1. Owen, J. J., M. D. Cooper, and M. C. Raff. 1974. In vitro generation of B lymphocytes in mouse fetal liver, a mammalian "bursa-equivalent". *Nature (Lond.).* 249:361.
2. Calvert, J. E., M. F. Kim, W. E. Gathings, and M. D. Cooper. 1983. Differentiation of B lineage cells from fetal liver of neonatal mice: generation of immunoglobulin isotype diversity in vitro. *J. Immunol.* 131:1693.

3. Abney, E. R., M. D. Cooper, J. F. Kearney, A. R. Lawton, et al. 1978. Sequential expression of immunoglobulin on developing mouse B lymphocytes: a systematic survey suggests a model for the generation of immunoglobulin isotype diversity. *J. Immunol.* 120:2041.
4. Gearhart, P. J., J. L. Hurwitz, and J. J. Cebra. 1980. Successive switching of antibody isotypes expressed within lines of a B cell clone. *Proc. Natl. Acad. Sci. USA.* 77:5424.
5. Mongini, P. K. A., K. E. Stein, and W. E. Paul. 1981. T cell regulation of IgG subclass antibody production in response to T independent antigens. *J. Exp. Med.* 153:1.
6. Mongini, P. K. A., W. E. Paul, and E. S. Metcalf. 1982. T cell regulation of immunoglobulin class expression in the antibody response to trinitrophenyl-Ficoll. *J. Exp. Med.* 155:884.
7. Torrigianni, G. 1972. Quantitative estimation of antibody in the immunoglobulin classes of the mouse. II. Thymic dependence of the different classes. *J. Immunol.* 108:161.
8. Rosenberg, Y. J., and J. M. Chiller. 1979. Ability of antigen-specific helper cells to effect a class-restricted increase in total Ig-secreting cells in spleens after immunization with the antigen. *J. Exp. Med.* 150:517.
9. Brittle, J. H., and H. L. Playfair. 1980. The role of T cells in regulating immunoglobulin class and subclass. *Immunology.* 41:743.
10. Braley-Mullen, H. 1974. Regulatory role of T cells in IgG antibody formation and immune memory to type III pneumococcal polysaccharide. *J. Immunol.* 113:1909.
11. Ivars, F., G. Nyberg, D. Holmberg, and A. Coutinho. 1983. Immune responses to bacterial dextrans. II. T cell control of antibody isotypes. *J. Exp. Med.* 158:1498.
12. Mongini, P. K. A., W. E. Paul, and F. S. Metcalf. 1983. IgG subclass, IgE and IgA anti-trinitrophenyl antibody production within trinitrophenyl-Ficoll-responsive B cell clones. Evidence in support of three distinct switching pathways. *J. Exp. Med.* 157:69.
13. Kiyono, H., M. D. Cooper, J. F. Kearney, L. M. Mosteller, S. M. Michalek, W. J. Koopman, and J. R. McGhee. 1984. Isotype specificity of helper T cell clones. Peyer's patch Th cells preferentially collaborate with mature IgA B cells for IgA responses. *J. Exp. Med.* 159:798.
14. Mayer, L., S. M. Fu, and H. G. Kunkel. 1982. Human T cell hybridomas secreting factors for IgA-specific help, polyclonal B cell activation, and B cell proliferation. *J. Exp. Med.* 156:1860.
15. Isakson, P. C., E. Pure, E. S. Vitteta, and P. H. Krammer. 1982. T cell derived B cell differentiation factor(s). Effect on isotype switch of murine B cells. *J. Exp. Med.* 155:734.
16. Kawanishi, H., L. Saltzman, and W. Strober. 1983. Mechanisms regulating IgA class-specific Ig production in murine gut-associated lymphoid tissues. I. T cells derived from Peyer's patches that switch sIgM B cells to sIgA B cells in vitro. *J. Exp. Med.* 157:433.
17. Kawanishi, H., L. Saltzman, and W. Strober. 1983. Mechanisms regulating IgA class-specific Ig production in murine gut-associated lymphoid tissues. II. Terminal differentiation of postswitch sIgA-bearing Peyer's patch B cells. *J. Exp. Med.* 158:649.
18. Douvas, A. S., W. E. Stumph, P. Reyes, and E. M. Tan. 1979. Isolation and characterization of nuclear ribonucleoprotein complexes using human anti-nuclear ribonucleoprotein antibodies. *J. Biol. Chem.* 254:3608.
19. Cruchaud, A., F. Rosen, J. Craig, C. A. Janeway, and D. Gitin. 1962. The site of the 19S γ-globulins in dysgammaglobulinemia. *J. Exp. Med.* 115:1141.
20. Gottleib, A. B., S. M. Fu, D. T. Y. Yu, C. Y. Wang, and H. G. Kunkel. 1979. The nature of the stimulatory cell in human allogeneic and autologous MLC reactions: role of isolated IgM-bearing cells. *J. Immunol.* 123:1497.

21. Bigler, R.D., D. E. Fisher, C. Y. Wang, E. A. Rinnooy Kan, and H. G. Kunkel. 1983. Idiotype-like molecules on cells of a human T cell leukemia. *J. Exp. Med.* 158:1000.
22. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting Ig of a given type or class. *Eur. J. Immunol.* 6:588.
23. Rosen, F., and C. A. Janeway. 1966. The gammaglobulines. III. The antibody deficiency syndromes. *N. Engl. J. Med.* 275:709.
24. Goldman, A., S. Ritzman, and E. Houston. 1967. Dysgammaglobulinemic antibody deficiency syndrome. Increased γM-globulins and decreased γG and γA-globulins. *J. Pediatr.* 70:16.
25. Geha, R. S., N. Hyslop, S. Alani, F. Farah, E. E. Schneeberger, and F. Rosen. 1979. Hyper-immunoglobulin M immunodeficiency (dysgammaglobulinemia). Presence of immunoglobulin M-secreting plasmacytoid cells in peripheral blood and failure of immunoglobulin M-immunoglobulin G switch in B cell differentiation. *J. Clin. Invest.* 63:868.
26. Levitt, D., P. Haber, K. Rich, and M. D. Cooper. 1983. Hyper IgM immunodeficiency. A primary dysfunction of B lymphocyte isotype switching. *J. Clin. Invest.* 72:1650.
27. Kuritani, T., and M. D. Cooper. 1982. Human B cell differentiation. I. Analysis of immunoglobulin heavy chain switching using monoclonal anti-immunoglobulin M, G, and A antibodies and pokeweed mitogen-induced plasma cell differentiation. *J. Exp. Med.* 155:839.
28. Stevens, R. H., E. Macy, and C. J. Thiele. 1981. Evidence that pokeweed mitogen-reactive B cells are pre-committed in vivo to the high rate secretion of a single immunglobulin isotype in vitro. *Scand. J. Immunol.* 14:449.
29. Davis, M. M., S. K. Kim, L. Hood. 1981. DNA sequences mediating class switching in α-immunoglobulins. *Science (Wash. DC).* 209:1360.