T LYMPHOCYTE-MEDIATED SUPPRESSION OF MYELOMA FUNCTION IN VITRO

III. Regulation of Antibody Production in Hybrid Myeloma Cells by T Lymphocytes*

BY ABUL K. ABBAS, STEVEN J. BURAKOFF, MALCOLM L. GEFTER, AND MARK I. GREENE

From the Departments of Pathology, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts 02115; and the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Downloaded from http://rup.silverchair.com/jem/article-pdf/152/4/969/1091067/969.pdf by guest on 02 June 2023

Humoral immune responses are known to be regulated, both positively and negatively, by antigen- and idiotype-specific T lymphocytes. Despite the large body of evidence supporting this notion (1–3), the mechanisms by which T cells influence B lymphocyte differentiation and function are largely unknown. One of the major reasons for this is the vast heterogeneity of the immune system, so that only very few lymphoid cells in an individual are committed to recognizing and responding to any defined antigenic determinant. As one approach to investigating the regulation of B lymphocytes and antibody secreting cells, we, as well as others, have focused on the development of antigen-specific myelomas as model systems for lymphoid cells because such homogeneous tumor lines are amenable to detailed biochemical and morphologic analyses (4, 5). It is now established that the function of myeloma cells can be regulated by tolerogenic antigens and immune complexes (6, 7), carrier-specific helper and suppressor T cells (8), idiotype-reactive T lymphocytes (9–11), and hapten-specific cytolytic T cells (12).

Recently, we have described two types of T cells that specifically suppress myeloma function in vitro. The first (10) is an idiotype-reactive suppressor T cell (Ts),[1] which is induced by immunizing BALB/c mice with syngeneic cells to which the BALB/c myeloma protein, M315 (IgA,λ_2), is covalently coupled. Such Ts inhibit antibody secretion by MOPC 315 cells in vitro in the absence of any antigen without affecting cell growth or viability; moreover, these T lymphocytes, similar to Ts in other systems, bear surface determinants encoded by the I-J subregion of the H-2 complex, their precursors are sensitive to cyclophosphamide, and they can be bound to plastic dishes coated with the M315 myeloma protein (10). The anti-idiotypic specificity of these Ts was established by their inability to bind to plastic dishes coated with M460 myeloma protein, which, like M315, binds 2,4-dinitrophenol (DNP) and 2,4,6-trinitrophenol (TNP) but does not share idiotypic determinants with M315. The second type of

* Supported by National Institutes of Health grants AI-14478, AI-16349, CA-14723, AI-16396, and AI-13357.

[1] *Abbreviations used in this paper:* ABA, *p*-azobenzenearsonate; CTL, cytolytic T lymphocyte(s); DNP, 2,4-dinitrophenol; ECDI, 1-ethyl 3-(3-diaminopropyl) carbodiimide; FCS, fetal calf serum; KLH, keyhole limpet hemocyanin; PFC, plaque-forming cell(s); TNP, 2,4,6-trinitrophenol; Ts, suppressor T cell(s).

J. EXP. MED. © The Rockefeller University Press · 0022-1007/80/10/0969/10 $1.00
Volume 152 October 1980 969–978

regulatory T cell (12) is a BALB/c cytolytic T lymphocyte (CTL) specific for the hapten, TNP, which inhibits antibody secretion by TNP-binding MOPC 315 cells in the presence of soluble TNP-coupled keyhole limpet hemocyanin (KLH). The interaction of CTL and TNP-binding myeloma targets is read out as functional suppression of the myeloma cells and is followed by a subsequent reduction in viable myeloma cell recovery, which suggests that the inhibition of antibody secretion is a prelytic effect of the T cells. These effector T lymphocytes, like classical hapten-reactive CTL described by other investigators (13), are Thy-1 and Ly-2 positive, hapten specific, radioresistant, and H-2 restricted; their precursors are insensitive to cyclophosphamide; and they do not bind to plastic dishes coated with TNP-proteins. Moreover, only myeloma cells that bind the TNP-protein to anti-TNP membrane receptors are suppressible by hapten-specific CTL (12). We have proposed that this system provides a model for further investigation of the interactions between H-2-restricted, antigen-specific T lymphocytes and antigen-specific targets such as B cells. In addition, these experiments support the possibility that hapten-reactive CTL can function as regulators of physiologic anti-hapten antibody responses (12).

To further analyze the mechanisms by which idiotype-specific Ts and hapten-reactive CTL affect myeloma cell function, we have examined their effects on a hybrid cell line generated by fusing MOPC 315 cells with the IgG_{2b},κ-secreting myeloma MPC 11. Our previous experiments have established the specificity of the T lymphocytes in terms of recognition, i.e., idiotype-reactive Ts recognize idiotypic determinants on myeloma proteins in the absence of exogenous antigen (10), whereas syngeneic hapten-reactive CTL recognize receptor-bound hapten apparently in association with surface determinants encoded in the H-2 complex (12). The use of the hybrid cell line now permits us to analyze whether the mechanisms of myeloma suppression by these distinctly different T lymphocytes are the same or not. The particular issue we have addressed is whether the interaction of a regulatory T lymphocyte with such a hybrid myeloma cell will affect production of one or both immunoglobulins (Ig) secreted by the hybrid. The major conclusion is that TNP-specific CTL, in the presence of TNP-KLH, suppress the secretion of both Ig by the hybrid cells, whereas idiotype-specific Ts inhibit secretion of only the Ig bearing the appropriate idiotype. The implications of these findings for the mechanism of action of regulatory T lymphocytes are discussed.

Materials and Methods

Cell Lines, Myeloma Proteins, and Antigens. BALB/c myelomas MOPC 315 (which secretes IgA,λ_2 antibody specific for DNP and TNP) and MPC 11 (IgG_{2b},κ of unknown specificity) were tissue culture-adapted lines that have been described in detail in previous publications (14, 15). By plaque assays, only 40–70% of the myeloma cells secrete Ig at any time (14, 15); whether this is related to the stage of the cell cycle or to the presence of distinct subpopulations within each myeloma line or to other factors is a question that is currently being investigated. Hybrids between these parental lines were derived by polyethylene glycol fusion and were selected and cloned on the basis of their ability to secrete one or both Ig isotypes (15). The MOPC 315-MPC 11 hybrid used in these studies has been referred to as the S001 line in earlier papers (15). This line secretes both IgG_{2b},κ and IgA,λ_2 molecules, with virtually all (>90%) κ-chains being IgG_{2b} associated, and the majority of the λ_2-chains being IgA associated (15). After subcloning, the rate of spontaneous loss of IgG_{2b},κ in the hybrid line is <1 in 10^5 cells per generation, and of IgA is <1 in 10^3 cells per generation (16). Analyses of cell-surface Ig in such hybrid lines by immunofluorescence and radioimmunoassays have shown expression of both Ig

Downloaded from http://rup.silverchair.com/jem/article-pdf/152/4/969/1091067/969.pdf by guest on 02 June 2023

isotypes in amounts similar to those seen in the parental lines (16) (G. Siebert and M. L. Gefter. Unpublished data.). All myeloma lines were maintained in suspension culture in Dulbecco's modified Eagle's medium with penicillin, streptomycin, nonessential amino acids, and 20% heat-inactivated fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, N. Y.) at 37°C in a humidified atmosphere of 5% CO_2. P815 (DBA/2 mastocytoma) cells were maintained in ascites form.

M315 protein was purified from ascites of tumor-bearing mice by ammonium sulfate precipitation (45% saturation) and affinity chromatography on DNP bovine serum albumin-coupled Sepharose 4B (10). MPC 11 protein was purified from tumor ascites by binding to staphylococcal protein A-Sepharose and elution with 0.1 M acetate buffer (pH 4). For some experiments this protein was further purified by ion-exchange chromatography on Sephadex QAE-A25 (17). Hapten (TNP) conjugates of KLH were prepared as described by Ballas and Henney (18). The conjugates used in these experiments were $TNP_{28}KLH$ and $TNP_{30}KLH$ (28 and 30 mol of TNP/100,000 mol wt of KLH).

Generation of Idiotype- and Hapten-reactive Lymphocytes. Ts reactive with myeloma idiotypes were generated as described previously (10). Briefly, 4×10^8 BALB/c spleen lymphocytes were coupled with 2 mg of M315 or MPC 11 protein, with 1-ethyl 3-(3-diaminopropyl) carbodiimide (ECDI) as the coupling reagent. Under these conditions, the coupling efficiency was 5-10%, and the cells were 60-80% viable by trypan blue dye exclusion. BALB/c mice were immunized intravenously with 5×10^7 viable myeloma protein-coupled or control (ECDI-treated) cells. Spleens and thymuses were removed 7 d later, freed of erythrocytes by treatment with Tris-buffered 0.83% NH_4Cl, and spleen cells were passed through nylon-wool columns to collect the nonadherent fraction (<7-10% surface Ig positive by immunofluorescence). Binding of idiotype-reactive Ts to myeloma protein-coated plastic dishes was done as previously described (10).

CTL specific for TNP were generated by in vitro stimulation of BALB/c spleen cells with 1,500-rad x-irradiated, TNP-modified syngeneic splenocytes (19). Cytolytic activity was measured by 4-h ^{51}Cr-release assays (19).

Myeloma Cell Cultures and Assays for Antibody Secretion. These techniques have been described in detail previously (10, 12). Viable myeloma cells were isolated by centrifugation over Ficoll-Isopaque, and 10^5 cells were cultured in triplicate with idiotype-reactive Ts or TNP-specific CTL in flat- or round-bottom microculture plates (Linbro Chemical Co., Hamden, Conn.), respectively. Cultures were done in total vol of 0.2 ml RPMI-1640 (Grand Island Biological Co.) with penicillin, streptomycin, 10% FCS, 10 mM Hepes, and 5×10^{-5} M 2-mercaptoethanol at 37°C in a humidified atmosphere of 5% CO_2. At the end of 1-5 d of culture, the cells were harvested, washed twice, and aliquots were assayed by reverse hemolytic plaque assays with protein A-coated sheep erythrocytes as indicator cells (10, 20). Preliminary experiments established the specificity of this assay: with rabbit anti-MPC 11 serum, only MPC 11 but not MOPC 315 plaque-forming cells (PFC) were detected, and the reverse was true with rabbit anti-M315 developing serum. After coculture with idiotype-reactive Ts, an aliquot of each culture was also assayed for viable myeloma cell recovery (viability being judged by trypan blue dye exclusion). Results are expressed as PFC $\times 10^{-3}$/culture, or PFC/10^3 recovered viable myeloma cells.

Results

Effects of Idiotype-reactive Ts on Myeloma Cells. Thymic and nylon-wool nonadherent splenic T cells from BALB/c mice immunized intravenously with M315- or MPC 11-coupled syngeneic splenocytes were cultured with the parent myeloma lines or the hybrid cells for 4 d, after which Ig secretion by the myeloma cells was measured. As shown in Table I, T lymphocytes from mice immunized with M315-coupled cells suppress antibody secretion by MOPC 315 but not MPC 11 cells, and vice versa. Moreover, when the hybrid cells are analyzed, M315-specific Ts inhibit IgA secretion only, whereas MPC 11-specific Ts suppress IgG secretion only. Pooled data from four experiments done with the hybrid line are shown in Table II, supporting this conclusion. Several points are noteworthy. First, comparable suppression is seen

Downloaded from http://rup.silverchair.com/jem/article-pdf/152/4/969/1091067/969.pdf by guest on 02 June 2023

TABLE I

Effect of Idiotype-reactive Ts on MOPC 315, MPC 11, and Hybrid Myeloma Cell Lines

Myeloma cells	10^6 thymocytes from mice injected intravenously with	IgA PFC: PFC × 10^{-3}/culture (mean ± SE)	IgA PFC: P*	IgA PFC: PFC/10^3 viable myeloma cells (mean ± SE)	IgA PFC: P	IgG PFC: PFC × 10^{-3}/culture (mean ± SE)	IgG PFC: P	IgG PFC: PFC/10^3 viable myeloma cells (mean ± SE)	IgG PFC: P
MOPC 315	None	40.3 ± 4.1	-	656 ± 41	-	0			
MOPC 315	M315 cells	17.9 ± 1.5	<0.01	363 ± 22	<0.005				
MOPC	MPC 11 cells	39.2 ± 8.2	NS‡	567 ± 142	NS				
MPC 11	None	0				71.9 ± 4.6		691 ± 45	
MPC 11	M315 cells					72.0 ± 4.8	NS	692 ± 47	NS
MPC 11	MPC 11 cells					47.5 ± 4.7	<0.05	371 ± 45	<0.01
MOPC 315-MPC 11 hybrid	None	43.6 ± 3.2		498 ± 37		47.9 ± 2.2	-	547 ± 25	-
MOPC 315-MPC 11 hybrid	M315 cells	20.9 ± 4.0	<0.05	248 ± 47	<0.05	51.3 ± 6.5	NS	607 ± 77	NS
MOPC 315-MPC 11 hybrid	MPC 11 cells	41.0 ± 4.6	NS	445 ± 50	NS	32.8 ± 3.3	<0.02	357 ± 36	<0.01

10^5 myeloma cells were cultured in triplicate with 10^6 thymocytes from mice injected intravenously 7 d previously with M315- or MPC 11-coupled splenocytes. After 4 d of culture at 37°C, the cells were washed, and aliquots were assayed for IgA and IgG PFC as well as viable myeloma cell recovery.

* Calculated by Student's t tests, with groups containing myeloma cells alone as controls. Groups showing significant inhibition ($P < 0.05$) are underlined.

‡ NS, not significant.

TABLE II

Effect of Idiotype-reactive Ts on MOPC 315-MPC 11 Hybrids

Suppressor cells: Intravenous immunization	Suppressor cells: Cells	IgA PFC: PFC × 10^{-3}/culture (mean ± SE)	IgA PFC: P*	IgA PFC: PFC/10^3 viable myeloma cells (mean ± SE)	IgA PFC: P	IgG PFC: PFC × 10^{-3}/culture (mean ± SE)	IgG PFC: P	IgG PFC: PFC/10^3 viable myeloma cells (mean ± SE)	IgG PFC: P
None		43.4 ± 3.1	-	459 ± 27	-	44.5 ± 2.1	-	486 ± 26	-
ECDI cells	1–2 × 10^6 thymus	38.1 ± 3.9	NS‡	362 ± 53	NS	43.6 ± 2.9	NS	403 ± 34	NS
ECDI cells	1–2 × 10^6 spleen	43.9 ± 6.9	NS	490 ± 99	NS	48.2 ± 3.2	NS	496 ± 20	NS
M315 cells	1–2 × 10^6 thymus	25.4 ± 2.0	<0.001	316 ± 26	<0.001	43.2 ± 3.2	NS	535 ± 38	NS
M315 cells	1–2 × 10^6 spleen	22.1 ± 3.0	<0.001	241 ± 41	<0.001	44.4 ± 3.5	NS	518 ± 34	NS
MPC 11 cells	1–2 × 10^6 thymus	43.8 ± 3.8	NS	556 ± 52	NS	31.3 ± 3.0	<0.005	391 ± 39	NS
MPC 11 cells	1–2 × 10^6 spleen	43.7 ± 5.2	NS	481 ± 50	NS	32.6 ± 1.7	<0.001	360 ± 21	<0.005
M315 cells	3 × 10^6 thymus	14.7 ± 1.9	<0.001	186 ± 25	<0.001	36.3 ± 2.3	NS	459 ± 30	NS
MPC 11 cells	3 × 10^6 thymus	28.8 ± 1.4	>0.03	335 ± 17	>0.03	28.1 ± 4.2	<0.005	327 ± 49	<0.02

10^5 MOPC 315-MPC 11 hybrid cells were cultured in triplicate with BALB/c thymic or nylon-wool nonadherent splenic T lymphocytes from mice that had been immunized intravenously 7 d previously with M315- or MPC 11-coupled or control (ECDI-treated) syngeneic splenocytes. After 4 d of culture at 37°C, the cells were washed and assayed for IgA and IgG PFC and viable myeloma cell recovery. Data are pooled from four experiments.

* Calculated by Student's t tests with cultures of hybrid myeloma cells alone as controls. Groups showing significant inhibition are underlined. With 3 × 10^6 thymocytes, MPC 11-reactive Ts do suppress IgA PFC ($P < 0.05 > 0.03$) but to a much lesser extent than M315-reactive Ts.

‡ NS, not significant.

whether the results are expressed as PFC/culture or PFC/10^3 viable recovered myeloma cells, which indicates that the Ts are not cytostatic or cytolytic. This is further supported by our findings that such suppressor cells do not significantly affect viable myeloma cell recovery after up to 5-d of coculture, and the suppressors are not lytic for myeloma targets by ^{51}Cr-release assays (data not shown). Second, intravenous immunization with splenocytes treated with ECDI in the absence of any myeloma protein does not generate suppressor cells (Table II). Third, the degree of suppression

Downloaded from http://rup.silverchair.com/jem/article-pdf/152/4/969/1091067/969.pdf by guest on 02 June 2023

generally observed is 40–60% for MOPC 315 cells, which is similar to previously published results (10), and 25–45% for MPC 11. Increasing the number of Ts does increase the degree of suppression, but with $\geq 3 \times 10^6$ idiotype-specific Ts or control cells, nonspecific effects are seen (Table II), and under no conditions have we observed 100% inhibition of Ig secretion. Whether these limits on the degree of inhibition of PFC are because only a proportion of the myeloma cells express high-density receptor Ig molecules, as we have proposed earlier (10), or because of other factors, is a question that is currently being explored. Finally, kinetic analyses have shown that significant suppression of both parent and hybrid cell lines requires 3–4 d of coculture with T lymphocytes, which is similar to published observations with the MOPC 315 parent line (10). Moreover, we have never observed an enhancement of PFC induced by idiotype-immune T cells in either parent or hybrid myeloma line (10).

To confirm the specificity of these Ts, thymic or splenic T cells from mice immunized intravenously with M315-coupled or MPC 11-coupled splenocytes were plated on plastic dishes coated with either myeloma protein. The nonadherent fraction was then cocultured with the hybrid myeloma cell line for 4 d, and antibody secretion was measured. As shown in Table III, M315-reactive Ts inhibit only IgA secretion, and this effect is lost by depleting the Ts on M315-coated but not MPC 11-coated dishes, and the reverse is true of MPC 11-reactive Ts. These experiments establish that the Ts recognize unique determinants of the appropriate myeloma proteins. Because isologous immunization with myeloma proteins induces anti-idiotypic immunity, we refer to the Ts as "idiotype specific," although we have not demonstrated whether they recognize unique determinants in the combining sites, framework regions, or other regions of the myeloma Ig.

Effects of Hapten-specific CTL on Myeloma Cells. BALB/c TNP-reactive CTL generated by in vitro stimulation and x-irradiated were cultured with MOPC 315, MPC 11, and hybrid cells with or without TNP-KLH. As shown in Table IV, coculture only in the presence of soluble antigen leads to inhibition of antibody secretion by MOPC 315 cells but not by MPC 11 cells, which lack membrane receptors for TNP.

TABLE III
Depletion of Idiotype-reactive Ts on Dishes Coated with Myeloma Proteins

2×10^6 suppressor cells		Percent inhibition (mean)	
Intravenous priming	Depleted on	IgA PFC	IgG PFC
M315 cells	None	37.4	11.8
M315 cells	M315-coated dish	2.3	ND*
M315 cells	MPC 11-coated dish	33.9	ND
MPC 11 cells	None	1.7	44.5
MPC 11 cells	M315-coated dish	ND	41.2
MPC 11 cells	MPC 11-coated dish	ND	8.7

10^5 MOPC 315-MPC 11 hybrid cells were cultured with 2×10^6 splenic or thymic T cells generated by intravenous immunization and treated as shown. After 4 d of culture, IgA and IgG PFC were assayed. Results are pooled from four experiments and normalized and expressed as percent inhibition of PFC per culture compared with control cultures of myeloma cells alone. Groups showing significant inhibition ($P < 0.05$) in each experiment are underlined.
* ND, not done.

Downloaded from http://rup.silverchair.com/jem/article-pdf/152/4/969/1091067/969.pdf by guest on 02 June 2023

TABLE IV

Effect of TNP-reactive CTL and TNP-KLH on MOPC 315, MPC 11, and Hybrid Cell Lines

Myeloma cells	TNP_{28}-KLH	3×10^5 effector cells	IgA PFC $\times 10^{-3}$/culture (mean ± SE)	P*	IgG PFC $\times 10^{-3}$/culture (mean ± SE)	P
MOPC 315	None	None	45.4 ± 2.6	–	0	
MOPC 315	50 μg/ml	None	35.1 ± 2.4	–		
MOPC 315	50 μg/ml	Anti-TNP CTL	18.3 ± 2.6	<0.001		
MOPC 315	None	Anti-TNP CTL	39.4 ± 3.6	NS‡		
MPC 11	None	None	0		62.1 ± 4.4	–
MPC 11	50 μg/ml	None			54.0 ± 2.9	–
MPC 11	50 μg/ml	Anti-TNP CTL			50.6 ± 3.4	NS
MPC 11	None	Anti-TNP CTL			60.1 ± 3.5	NS
MOPC 315-MPC 11 hybrid	None	None	40.5 ± 11.6	–	37.6 ± 1.6	–
MOPC 315-MPC 11 hybrid	50 μg/ml	None	44.7 ± 2.7	–	38.0 ± 2.3	–
MOPC 315-MPC 11 hybrid	50 μg/ml	Anti-TNP CTL	26.8 ± 0.9	<0.001	22.0 ± 0.3	<0.001
MOPC 315-MPC 11 hybrid	None	Anti-TNP CTL	34.5 ± 3.1	NS	32.1 ± 2.8	NS

^{51}Cr-labeled target	Assays for cytolysis of TNP-modified and unmodified ^{51}Cr-labeled targets by TNP-reactive CTL revealed the following in one representative experiment: specific lysis by anti-TNP CTL at effector:target ratio of:	
	20:1	80:1
TNP-modified P815	58.0	82.9
Unmodified P815	6.7	21.0
TNP-modified MOPC 315	91.2	98.3
Unmodified MOPC 315	19.6	27.0
TNP-modified MPC 11	63.6	69.0
Unmodified MPC 11	0	8.6
TNP-modified MOPC 315-MPC 11 hybrid	58.6	95.1
Unmodified MOPC 315-MPC 11 hybrid	0	10.7

10^5 myeloma cells were cultured in triplicate with and without TNP_{28}KLH and 3×10^5 1,500-R x-irradiated TNP-reactive CTL generated by in vitro stimulation. At the end of 24 h at 37°C, the cells were washed three times, and aliquots were assayed for IgA and IgG PFC.

* Calculated by Student's *t* tests, with groups containing myeloma cells and the equivalent concentration of antigen but lacking CTL as controls. Groups showing significant inhibition are underlined. Data are pooled from two representative experiments.

‡ NS, not significant.

Hybrid cells show inhibition of both IgA and IgG secretion, which is strikingly different from the effects of idiotype-specific Ts. In the absence of antigen, TNP-specific CTL have no significant effect. Similar results have been obtained with 10–100 μg/ml of TNP-KLH. Moreover, all three tumor cell lines can be lysed by TNP-specific CTL if the targets are directly haptenated (Table IV), but no lysis is seen in 4-h ^{51}Cr-release assays with unmodified targets alone (Table IV) or unmodified targets incubated with soluble antigen (data not shown). Finally, when MOPC 315 or hybrid cells are cocultured with TNP-KLH and 1,500-R x-irradiated anti-TNP CTL for 24

Downloaded from http://rup.silverchair.com/jem/article-pdf/152/4/969/1091067/969.pdf by guest on 02 June 2023

h, washed, and aliquots recultured in fresh medium, after 2–3 d the viable myeloma cell recovery is reduced by 25–50% compared with control cultures of myeloma cells alone. This suggests that the functional inactivation of myeloma targets is followed by target-cell death.

Discussion

These experiments show that the function of an antigen-binding hybrid myeloma cell line can be altered in strikingly different ways by different types of regulatory T lymphocytes. Thus, hapten-reactive CTL, in the presence of soluble hapten proteins, inhibit the secretion of both Ig by the hybrid myeloma line, whereas idiotype-reactive Ts suppress secretion of only the Ig bearing the relevant idiotype.

The finding of highly specific, differential suppression of Ig secretion by anti-idiotypic Ts has important implications for the mechanisms of action of such regulatory T cells. It should be pointed out that the rate of spontaneous loss of expression of one Ig by the hybrid line is so low (16) that such differential suppression cannot be accounted for by any form of spontaneous loss of production of one Ig during culture. These experiments establish the specificity of idiotype-reactive Ts at two levels. First, suppressor cells induced by intravenous immunization with M315- or MPC 11-coupled syngeneic splenocytes are clearly specific in terms of recognition, because they inhibit only the appropriate parent myeloma line (Table I) and bind to the appropriate myeloma protein (Table III). Second, each idiotype-reactive Ts population inhibits the secretion of only the Ig bearing that idiotype by the hybrid line (Tables I and II). This selective effect of the Ts on the function of hybrid myeloma cells can be accounted for by one of two mechanisms. It is conceivable that suppression is a surface phenomenon, perhaps related to the binding of an anti-idiotypic factor released from Ts to the myeloma cells, which results in a secretory blockade or absorption of the secreted idiotype. Such a mechanism is supported by the studies of Rohrer et al. (9), who have found that MOPC 315 cells enclosed within diffusion chambers and implanted in the abdomens of M315 idiotype-immune BALB/c mice show an idiotype-specific secretory blockade that is reversed by treating the myeloma cells with pronase. In our in vitro system, pronase treatment fails to reverse the inhibition of myeloma cells that have been cocultured with idiotype-reactive Ts (data not shown). The alternative possibility is that the inhibitory signal delivered by idiotype-specific Ts affects only that portion of the Ig-producing apparatus of the cell that is related or linked to the surface idiotype. The intracellular sequence of events that could lead to such an effect is unknown. Attempts are currently under way to develop experimental conditions for inducing maximal idiotype-specific inhibition of myeloma cells in vitro so that the molecular basis of this inhibition can be analyzed. Nevertheless, irrespective of the precise mechanism, these results indicate that the effector function of idiotype-reactive Ts is highly specific in that it can affect one but not another highly specialized function of an appropriate target. Moreover, it is also clear that in this system, functional inactivation is not accompanied by cell death. We do not know if classical, nonlytic, antigen-specific Ts will have an effect similar to idiotype-specific Ts. Experiments are currently in progress to determine if hapten- or carrier-reactive Ts will alter myeloma function in vitro in the presence of hapten-carrier conjugates.

The observation that syngeneic anti-TNP CTL, in the presence of TNP-KLH,

Downloaded from http://rup.silverchair.com/jem/article-pdf/152/4/969/1091067/969.pdf by guest on 02 June 2023

inhibit the secretion of both IgA and IgG by the MOPC 315-MPC 11 hybrid line (Table IV) is consistent with earlier results that suggest that functional suppression represents a prelytic effect of these T lymphocytes (12). This view is supported by experiments done by ourselves and others (21–23) showing that functional inactivation of myeloma and mastocytoma targets is a highly sensitive assay for classical, alloreactive CTL, and appears to reflect a prelytic abnormality induced by the CTL. The failure of anti-TNP CTL to interact with MPC 11 cells has been reported earlier, and, taken together with experiments utilizing double conjugates of TNP and *p*-azobenzenearsonate (ABA) with KLH and ABA-reactive CTL, indicates that only antigen bound to specific membrane Ig receptors renders the myeloma targets suppressible by hapten-reactive CTL (12). Thus, once TNP-KLH is bound to anti-TNP receptors on the MOPC 315-MPC 11 hybrid cells, syngeneic TNP-reactive CTL interact with these cells and inhibit production of both Ig isotypes. The subsequent reduction of viable myeloma cell recovery after more prolonged culture in fresh medium would indicate that such suppressed myeloma targets are probably committed to lyse.

Finally, the relevance of these experiments to T cell regulation of physiologic humoral immune responses must be considered. We, as well as others, have presented experimental evidence supporting the concept that myeloma cells are valid model systems for analyzing lymphocyte function (4, 5), although it is unclear to what extent a fusion-derived hybrid myeloma line differs from parental lines. Nevertheless, the studies reported in this paper suggest that regulation of B cell function might occur by one of two mechanisms. Idiotype-reactive and, possibly, antigen-reactive Ts that are nonlytic can inhibit immunocompetent cells expressing the appropriate receptors on their surface without irreversibly injuring these cells. Such a mechanism would represent a form of clonal inhibition. In contrast, antigen-reactive CTL, which may also play a role in regulating humoral immunity (12), would accomplish this by clonal deletion. Whether such mechanisms can actually be distinguished in different kinds of physiologic immune responses and their regulation remains to be answered.

Summary

To investigate the mechanisms by which T lymphocytes regulate myeloma function in vitro, the effects of regulatory T cells on antibody secretion by a hybrid myeloma cell line were examined. Suppressor T cells (Ts) specific for idiotypic determinants on M315 (IgA,λ_2 anti-2,4-dinitrophenol and anti-2,4,6-trinitrophenol [TNP]) and MPC 11 (IgG_{2b},κ) myeloma proteins inhibit antibody secretion by the appropriate parental myeloma cells. When cocultured with a hybrid cell line derived by fusion of MOPC 315 and MPC 11 myelomas, the idiotype-reactive Ts inhibit secretion of only the immunoglobulin (Ig) bearing the relevant idiotype. In contrast, syngeneic TNP-reactive cytolytic T lymphocytes (CTL) inhibit antibody secretion by TNP-binding MOPC 315 cells but not by MPC 11 cells in the presence of soluble TNP-keyhole limpet hemocyanin (KLH), and this inhibition probably represents a prelytic effect of the CTL. Such TNP-reactive CTL, in the presence of TNP-KLH, inhibit both IgA and IgG secretion by the MOPC 315-MPC 11 hybrid, which is consistent with a prelytic effect. Thus, myeloma hybrids are a useful tool for investigating the effector function of regulatory T cells. These results are discussed with reference to the mechanisms of action of regulatory T cells and their relevance to modulation of

Downloaded from http://rup.silverchair.com/jem/article-pdf/152/4/969/1091067/969.pdf by guest on 02 June 2023

physiologic humoral immune responses.

We thank Roxane Barfknecht, John Riccio, and Kathy Vanasse for their excellent technical assistance, and Ms. Elissa Faulkner for invaluable secretarial assistance.

Received for publication 28 April 1980 and in revised form 23 June 1980.

References

1. Gershon, R. K. 1974. T cell control of antibody production. *Contemp. Top. Immunobiol.* **3:**1.
2. Eichmann, K. 1978. Expression and function of idiotypes on lymphocytes. *Adv. Immunol.* **26:** 195.
3. Greene, M. I., M. S. Sy, A. Nisonoff, and B. Benacerraf. The genetic and cellular basis of antigen and receptor stimulated regulation. *Mol. Immunol.* In press.
4. Abbas, A. K. 1979. Antigen and T lymphocyte mediated suppression of myeloma cells: model systems for regulation of lymphocyte function. *Immunol. Rev.* **48:**245.
5. Lynch, R. G., J. W. Rohrer, B. Odermatt, H. D. Gebel, J. R. Autry, and R. G. Hoover. 1979. Immunoregulation of murine myeloma cell growth and differentiation: a monoclonal model of B cell differentiation. *Immunol. Rev.* **48:**45.
6. Abbas, A. K., and G. G. B. Klaus. 1977. Inhibition of antibody production in mouse plasmacytoma cells by antigens. *Eur. J. Immunol.* **7:**667.
7. Abbas, A. K., and G. G. B. Klaus. 1978. Antigen-antibody complexes suppress antibody production by mouse plasmacytoma cells *in vitro. Eur. J. Immunol.* **8:**217.
8. Rohrer, J. W., and R. G. Lynch. 1977. Specific, immunologic regulation of differentiation of immunoglobulin expression in MOPC-315 cells during *in vivo* growth in diffusion chambers. *J. Immunol.* **119:**2045.
9. Rohrer, J. W., B. Odermatt, and R. G. Lynch. 1979. Immunoregulation of murine myeloma: isologous immunization with M315 induces idiotype-specific T cells that suppress IgA secretion by MOPC-315 cells *in vivo. J. Immunol.* **122:**2011.
10. Abbas, A. K., L. L. Perry, B. A. Bach, and M. I. Greene. 1980. Idiotype-specific T cell immunity. I. Generation of effector and suppressor T lymphocytes reactive with myeloma idiotypic determinants. *J. Immunol.* **124:**1160.
11. Flood, P. M., C. Phillips, M. A. Taupier, and H. Schreiber. 1980. Regulation of myeloma growth *in vitro* by idiotype-specific T lymphocytes. *J. Immunol.* **124:**424.
12. Abbas, A. K., S. E. Ratnofsky, and S. J. Burakoff. 1980. T lymphocyte-mediated suppression of myeloma function in vitro. II. Evidence for regulation of hapten-binding myelomas by syngeneic hapten-specific cytolytic T lymphocytes. *J. Exp. Med.* **152:**306.
13. Shearer, G. M., and A. M. Schmitt-Verhulst. 1977. Major histocompatibility complex restricted cell-mediated immunity. *Adv. Immunol.* **25:**55.
14. Sonensheim, G. E., M. Siekevitz, G. R. Siebert, and M. L. Gefter. 1978. Control of immunoglobulin secretion in the murine plasmacytoma line MOPC 315. *J. Exp. Med.* **148:** 301.
15. Siebert, G. R., J. F. Harris, and M. L. Gefter. 1978. Regulation of immunoglobulin biosynthesis in the murine plasmacytoma MOPC 315. *J. Immunol.* **121:**1808.
16. Siebert, G. R. 1978. Regulation of immunoglobulin biosynthesis in murine plasmacytomas and their derivatives. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, Mass.
17. Unkeless, J. C., and H. N. Eisen. 1975. Binding of monomeric immunoglobulins to Fc receptors of mouse macrophages. *J. Exp. Med.* **142:**1520.
18. Ballas, Z. K., and C. S. Henney. 1979. Generation of H-2 restricted cytotoxic T cells by trinitrophenylated proteins *in vitro*: specificity and requirements. *J. Immunol.* **123:**1696.
19. Burakoff, S. J., R. N. Germain, M. E. Dorf, and B. Benacerraf. 1976. Inhibition of cell

Downloaded from http://rup.silverchair.com/jem/article-pdf/152/4/969/1091067/969.pdf by guest on 02 June 2023

mediated cytolysis of trinitrophenyl derivatized target cells by alloantisera directed at products of the K and D loci of the H-2 complex. *Proc. Natl. Acad. Sci. U. S. A.* **73:**625.

20. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting immunoglobulin of a given type or class. *Eur. J. Immunol.* **6:**588.
21. Abbas, A. K. 1979. T lymphocyte mediated suppression of myeloma function *in vitro.* I. Suppression by allogeneically activated T lymphocytes. *J. Immunol.* **123:**2011.
22. Watanabe, T., C. G. Fathman, and A. Coutinho. 1977. Clonal growth of T cells *in vitro*: preliminary attempts to a quantitative approach. *Immunol. Rev.* **35:**3.
23. Tsoukas, C. D., W. J. Wechter, and E. Martz. 1979. Evidence against Ca^{++} poisoning by killer cells: mast cells killed by T lymphocytes do not secrete prelytically. *J. Supramol. Struct.* **3:**(Suppl.):331. (Abstr.)

Downloaded from http://rup.silverchair.com/jem/article-pdf/152/4/969/1091067/969.pdf by guest on 02 June 2023