IMMUNOREGULATION OF
MURINE MYELOMA IN VITRO

II. Suppression of MOPC-315 Immunoglobulin Secretion and
Synthesis by Idiotype-specific Suppressor T Cells*

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A considerable body of experimental evidence (1) supports the Jerne hypothesis (2) that idiotypes are important in the autoregulation of B cells. Although the original concept was limited to regulatory antibodies that recognized individually specific determinents (idiotypes) located in the variable regions of other immunoglobulin molecules, subsequent studies (3–6) showed that certain T cells also recognized idiotypes. Anti-idiotypic T cells have been shown to include helper and suppressor cells that regulate individual B cell clones.

It has been difficult to develop a precise understanding of the events that occur in a B cell when it is regulated by anti-idiotypic T cells or antibodies because in conventional immune cell samples, the specifically regulated B cells comprise a minute fraction of the sample that is being studied. To circumvent this limition, we have studied the 2,4,6-trinitrophenyl[1] (TNP)-specific BALB/c plasmacytoma MOPC-315; it is a monoclonal B cell population that is responsive to idiotype-specific and TNP-antigen-specific immunoregulatory signals (7).

Previous studies identified three idiotype (Id^{315})-specific events that followed immunization of BALB/c mice with the IgA anti-TNP antibody (M315) produced by MOPC-315: (a) induction of anti-idiotypic (a-Id^{315}) antibodies predominantly of the IgG_1 subclass (8), (b) development of a-Id^{315} suppressor T cells (9, 10), and (c) establishment of Id^{315}-specific protection from challenge with lethal numbers of MOPC-315 cells (11). Subsequent studies of MOPC-315 cells enclosed in peritoneal diffusion chambers (9) suggested that: (a) anti-Id^{315} antibodies inhibited surface

Abbreviations used in this paper: a, anti; C′, guinea pig complement; Id^{315}, idiotypic antigens located in the variable regions of M315; Id^{460}, idiotypic antigens located in the variable regions of M460; M315, trinitrophenyl-binding $IgA\lambda_2$ protein produced by MOPC-315; M460, trinitrophenyl-binding IgAκ protein produced by MOPC-460; mRNA, messenger RNA; NP40, Nonidet P-40; PBS, phosphate-buffered saline; PFC, plaque-forming cell; RA315, mildly reduced and alkylated affinity-purified M315; RA460, mildly reduced and alkylated affinity-purified M460; TCA, trichloroacetic acid; TNP, 2,4,6-trinitrophenyl.

membrane expression of M315, and (b) anti-Id[315] suppressor T cells inhibited secretion of M315. Recently, we showed that anti-Id[315] antibodies inhibited surface membrane expression of M315 in vitro without influencing M315 secretion or MOPC-315 growth or viability.[2]

In this paper, an in vitro system was used to more precisely analyze the mechanism of inhibition of M315 secretion mediated by a-Id[315] suppressor T cells. These studies have established that a-Id[315] suppressor T cells (a) are Lyt-1⁻2⁺ cells that express a surface membrane binding site specific for Id[315], (b) mediate suppression via an Id[315]-specific diffusable product, (c) act directly on M315-secreting cells, and (d) inhibit M315 secretion by a selective and reversible inhibition of M315 biosynthesis.

Materials and Methods

Chemicals and Reagents. 2,4,6-trinitrobenzene sulfonate, protease type VI, and Sepharose 6MB were obtained from Sigma Chemical Co., St. Louis, MO. Sheep erythrocytes were purchased from Colorado Serum Co., Denver, CO. Rabbit anti-mouse IgA serum was obtained from Gateway Immunosera, St. Louis, MO, and guinea pig complement was obtained from Grand Island Biological Co., Grand Island, NY. Fetal bovine serum (lot 30351) was purchased from KC Biologicals, Lenexa, KS.

Mice. BALB/c mice were purchased from West Seneca Laboratories, West Seneca, NY. The mice were offered, *ad lib.*, a standard pellet chow and tap water containing 60 mg/liter aureomycin (American Cyanimid Corp., Pearl River, NY).

Plasmacytomas and Myeloma Proteins. An in vitro adapted line of MOPC-315 was established from an ascites tumor and had been maintained in continuous culture for ~3 yr at the time of these studies. The cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate, essential and nonessential amino acids, 25 mM Hepes, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 5×10^{-5} M 2-mercaptoethanol. Greater than 99% of these cells express cytoplasmic IgA detected by immunofluorescence, 80–90% express surfce M315 that is visualized with a TNP-sheep erythrocyte rosette assay (12), and 40–50% secrete sufficient M315 to form an anti-TNP plaque-forming cells (PFC) in an indirect Jerne assay (13). The cells were fed three times a week and split to ~5 × 10⁵ viable cells/ml after reaching a density of ~1.5 × 10⁶cells/ml.

M315 and M460 were purified as described previously (11).

Induction and Isolation of Idiotype-specific T Cells. BALB/c mice were hyperimmunized with a mildly reduced and alkylated form of M315 (RA315) in six injections at 1-wk intervals of 200 µg in 0.2 ml, as previously described (9). 3 d before killing, the hyperimmunized mice were boosted intravenously with 50 µg of RA315 in phosphate-buffered saline (PBS). Spleen cells were collected aseptically and erythrocytes lysed with NH₄-Cl-Tris, pH 7.4, and then passed through a nylon wool column (typically, 1.6 × 10⁸ cells/g of dry nylon-wool). The nylon wool-nonadherent cells were collected, subjected to various specific cell separation protocols (see below), and added to MOPC-315 cells at various T cell:MOPC-315 cell ratios.

The co-cultures were harvested after 24 h of incubation at 37°C. MOPC-315 cells were counted and their viability determined by trypan blue dye exclusion. MOPC-315 cells expressing surface membrane M315 were quantitated as TNP-rosette-forming cells, and M315-secreting cells were quantitated as TNP-PFC.

Co-Cultures and Marbrook-like Cultures. 1 × 10⁵ MOPC-315 cells were mixed with various numbers of either RA315-immune T cells or normal T cells and were incubated at 37°C in 1.0-ml cultures in 24-well culture plates (Costar 3524; Costar, Data Packaging, Cambridge, MA). In some experiments 0.2-ml co-cultures were incubated in 96-well flat-bottomed plates (Costar 3596). In experiments with Marbrook-like cultures, 1 × 10⁵ MOPC-315 cells were placed in the upper chamber (PC-2; Adaps, Inc., Dedham, MA) and separated from the lower chamber by

a 0.22-μm membrane (Millipore Corp., Bedford, MA). The lower chamber containing the T cells was formed by the well of a 24-well plate as described by Ullrich and Zolla-Pazner (14).

Cell Separation by Affinity Chromatography.   Immunoadsorbent columns were prepared by coupling RA315, RA460, monoclonal anti-Lyt-1.2 (clone 53-7.313), or monoclonal anti-Lyt-2.2 (clone 53-6.72) to Sepharose 6MB using the procedure described elsewhere (15). (Clones 53-7.313 and 53-6.72 were obtained from Noel Warner, University of New Mexico School of Medicine, Albuquerque, NM.) Briefly, 2-5 mg of each antibody was added to each milliliter of cyanogen bromide-activated Sepharose, achieving a coupling efficiency of >90%. 1-ml antibody-coupled gel columns were constructed using a T-B syringe (B-D, Rutherford, NJ) containing a nylon-wool plug. After washing with RPMI 1640 medium supplemented with 10% fetal calf serum and 10 mM Hepes, 1-2 $\times$ 10^7 nylon wool-nonadherent T cells were added to each column, incubated for 30 min at room temperature, and then nonadherent cells were gently eluted. In one set of experiments, the cells bound to RA315-Sepharose 6MB were eluted with soluble RA315 in PBS at a concentration of 2.0 mg/ml. In all other experiments the nonadherent cells were used.

[^{3}H]Leucine Incorporation Studies.   Incorporation of [^{3}H]leucine (76 Ci/mmol; Amersham Corp., Arlington Heights, IL) into M315 or total cell protein was assayed by culturing the MOPC-315 cells with 50 μCi of the isotope in each milliliter of leucine-free minimum essential medium (Grand Island Biological Co.) supplemented with 10% fetal calf serum.

Co-cultures of myeloma cells and T cells were incubated for 24 h, after which they were washed and pulsed with isotope. At harvest, the amount of [^{3}H]leucine incorporated into secreted M315 was quantitated by specific immunoprecipitation of the supernatant by the procedure described elsewhere (16). The intracellular M315 was similarly immunoprecipitated after a 30-min treatment of the washed cells with 0.5% nonidet P-40 (NP40) (Jet Propulsion Laboratory, Pasadena, CA) and removal of subcellular organelles by microcentrifugation. To correct for the radioactivity nonspecifically bound in the immunoprecipitates, control precipitates of normal rabbit γ-globulins and goat anti-rabbit globulin serum were formed in the labeled cell extracts or supernatants and processed along with experimental groups. This level of nonspecifically bound radioactivity (which was ~1% of the total counts added) was subtracted from each experimental group.

The same procedures were used for [^{3}H]leucine incorporation studies in the Marbrook culture system, with the exception that 48 h elapsed before the 24-h pulse with the isotope. In addition, cold 5% trichloroacetic acid (TCA) precipitation was used to quantitate the amount of [^{3}H]-leucine incorporated into total intracellular protein in MOPC-315 cells.

Data Analysis.   Each experiment was performed a minimum of three times and the data reported as the mean $\pm$ 1 SEM.

Results

Inhibition of M315 Secretion by Id^{315}-specific T Cells In Vitro.   Previous studies (9) showed that spleen cells from Id^{315}-immune mice could adoptively transfer inhibition of M315 secretion to normal mice and that the transfer was abrogated by treating the spleen cells with anti-θ (rabbit anti-mouse brain antisera) and complement before transfer. Therefore, nylon wool-purified splenic T lymphocytes from Id^{315}-immune mice were co-cultured at various concentrations with MOPC-315 cells in vitro. The data, shown in Table I, show that after a 24-h co-culture with the Id^{315}-immune spleen cells, there was no effect on MOPC-315 viability or surface membrane expression of M315 when compared with control cultures that contained normal splenic T cells mixed with MOPC-315 cells. In contrast, Id^{315}-immune T cells inhibited the frequency of M315-secreting cells by >90% compared with controls when the ratio of immune effector T cells to MOPC-315 cells was 200:1. As shown in Table II, a direct relationship exists between the dose of immune effector T cells added to the MOPC-315 cells and the level of inhibition of secretion. Almost total inhibition of secretion was observed at an 800-fold excess of Id^{315}-immune T cells, whereas only

TABLE I

Effect of Id^{315}-specific T Cells on MOPC-315 Cells In Vitro

Effector:MOPC-315 cell ratio*	Effector cells	Viability	Percentage of MOPC-315 with surface M315	Percentage of MOPC-315 cells that secrete M315
MOPC-315 cells alone	None	93 ± 2‡	89 ± 2	44 ± 3
50:1	Normal spleen T cells	86 ± 3	89 ± 2	41 ± 5
100:1	Normal spleen T cells	85 ± 5	84 ± 3	40 ± 4
200:1	Normal spleen T cells	83 ± 5	82 ± 3	38 ± 3
50:1	RA315-immune spleen T cells	78 ± 3	82 ± 4	36 ± 4
100:1	RA315-immune spleen T cells	78 ± 5	79 ± 5	8 ± 2
200:1	RA315-immune spleen T cells	76 ± 2	77 ± 5	3 ± 2

* MOPC-315 cell number was typically 10^5 viable cells/ml, and the effector cell number was adjusted according to the ratio indicated.
‡ Mean ± 1 SEM for three experiments.

TABLE II

Dose-dependent Suppression of M315 Secretion

Effector:MOPC-315 cell ratio*	Effector cells	Percentage of MOPC-315 cells that secrete M315
MOPC-315 cells alone	None	43 ± 3‡
100:1	Normal spleen T cells	41 ± 3
400:1	Normal spleen T cells	39 ± 2
800:1	Normal spleen T cells	36 ± 3
25:1	RA315-immune spleen T cells	41 ± 4
50:1	RA315-immune spleen T cells	32 ± 3
100:1	RA315-immune spleen T cells	12 ± 5
200:1	RA315-immune spleen T cells	8 ± 3
400:1	RA315-immune spleen T cells	3 ± 1
800:1	RA315-immune spleen T cells	2 ± 1

* MOPC-315 cell number was held constant at 10^5 viable cells/ml.
‡ Mean ± 1 SEM for three experiments.

minimal effects were observed when equivalent numbers of normal splenic T cells were added to MOPC-315 cultures.

Characterization of the Id^{315}-specific Effector Cell. Although inhibition of M315 secretion by nylon wool-passed cells implied that the effector cell was a T cell, more direct evidence was sought. Treatment of Id^{315}-immune spleen cells with anti-θ + guinea pig complement (C') eliminated the secretory suppression, whereas normal rabbit serum + C' had no effect (data not shown).

To determine the Lyt phenotype of the effector T cell, RA315-immune splenic T cells were applied to either anti-Lyt-1 or anti-Lyt-2 columns. The nonadherent cells from each column were then co-cultured with MOPC-315 cells. The results, shown in Table III, show that cells that passed through the anti-Lyt-1 column still retained

TABLE III

Id^315-specific T Suppressor Cells Are Lyt-1^-2^+

Effector:MOPC-315 cell ratio	Treatment of added RA315-Immune T cells	Percentage of MOPC-315 cells that secrete M315
MOPC-315 cells alone	None	55 ± 1*
50:1	No treatment	43 ± 1
100:1	No treatment	8 ± 1
50:1	Lyt-2.2 depletion‡	54 ± 2
100:1	Lyt-2.2 depletion	49 ± 3
50:1	Lyt-1.2 depletion§	14 ± 2
100:1	Lyt-1.2 depletion	6 ± 1
50:1	Lyt-2.2 depletion and	19 ± 3
50:1	Lyt-1.2 depletion‖	

* Mean ± 1 SEM for three experiments.
‡ Effector cells were depleted by passage over an anti-Lyt-2.2-Sepharose 6MB column.
§ Effector cells were depleted by passage over an anti-Lyt-1.2-Sepharose 6MB column.
‖ Equal numbers of nonadherent cells from each column were mixed and used as effectors.

their suppressive activity, whereas the anti-Lyt-2 column depleted the suppressor activity. Taken together, these data establish the phenotype of the effector T cells as Lyt-1$^-$2$^+$. Furthermore, when equal numbers of Lyt-1-depleted and Lyt-2-depleted cells were mixed and added to MOPC-315 cultures, the level of suppression observed was the same as with the same number of Lyt-1-depleted cells alone. This result suggests that (a) Lyt-1$^+$ cells did not have any augmenting or inhibiting effect on the Lyt-2$^+$ effectors, and (b) the Lyt-1$^+$23$^+$ cells, which were depleted by passage through either column in these mixing experiments, were not the effectors and were not required at the effector stage of M315 secretory inhibition. Furthermore, we also established that macrophages were not required at the effector stage (data not shown).

To investigate the idiotype specificity of secretory inhibition, columns of Sepharose 6MB conjugated with RA315 or RA460 were prepared. RA460 is the mildly reduced and alkylated IgA anti-TNP myeloma protein, M460, produced by MOPC-460. M315 and M460 have dissimilar idiotypes. RA315-immune splenic T cells were applied to the columns, and the nonadherent cells from each column were tested for suppressive activity on MOPC-315 cells. The data (Table IV) show that the T cells that mediated inhibition of M315 secretion bound to the RA315-Sepharose but not to the RA460-Sepharose column. Furthermore, T cells eluted from the RA315-Sepharose column with soluble RA315 at a concentration of 2 mg/ml were enriched in suppressor activity. Significant suppression was observed at a T cell:MOPC-315 cell ratio of only 12:1, and inhibition was virtually total at higher ratios. Few, if any, cells were retained by the RA460-Sepharose column, and elution with soluble RA315 failed to release cells. When soluble M315 (100 μg/ml) was added to the cultures, it prevented the T cells from suppressing M315 secretion (data not shown). These observations demonstrate that Id^{315}-specific suppressor T cells have surface recognition

TABLE IV

Depletion and Enrichment of Id^{315}-specific T Cells

Effector:MOPC-315 cell ratio	Treatment of added RA315 immune T cells	Percentage of MOPC-315 cells that secrete M315
MOPC-315 cells alone	No treatment	43 ± 3*
50:1	No treatment	12 ± 3
100:1	No treatment	8 ± 3
200:1	No treatment	3 ± 1
50:1	Id^{460} absorption‡	16 ± 4
100:1	Id^{460} absorption	10 ± 5
200:1	Id^{460} absorption	7 ± 2
50:1	Id^{315} absorption§	35 ± 3
100:1	Id^{315} absorption	36 ± 5
200:1	Id^{315} absorption	34 ± 6
12:1	Eluted from Id^{315} absorbant‖	11 ± 5
25:1	Eluted from Id^{315} absorbant	4 ± 4
50:1	Eluted from Id^{315} absorbant	1 ± 1

* Mean ± 1 SEM for three experiments.
‡ RA315-immune spleen T cells were passed over an Id^{460}-Sepharose 6MB column, and the nonadherent cells were used as effectors.
§ RA315-immune spleen T cells were passed over an Id^{315}-Sepharose 6MB column, and the nonadherent cells were used as effectors.
‖ Cells that adhered to the Id^{315}-Sepharose 6MB column were eluted with soluble RA315 in PBS at a concentration of 2 mg/ml.

structures that can (a) bind to M315 idiotypes and (b) distinguish between M315 and M460 idiotypes.

Kinetics of Inhibition of M315 Secretion. All the previous experiments measured M315 secretion after MOPC-315 cells had been co-cultured with the suppressor T cells for 24 h. To determine the kinetics of inhibition, we determined the frequency of secretory cells at 2-h intervals after the addition of RA315-immune splenic T cells to MOPC-315 cell cultures. Fig. 1 shows that inhibition of M315 secretion was first detected after 6 h of co-culture and progressed until an inhibition of 80–90% of the control PFC frequency was observed at 24 h. This result suggests that the regulatory T cells were already programmed for the suppressive activity when they were added to the culture and probably did not require further proliferation or differentiation. Furthermore, because MOPC-315 cells were secreting M315 at the time that the suppressor cells were added to the culture, the suppressor T cells acted directly on the actual antibody-secreting cell rather than on a precursor to the antibody-secreting cell.

Inhibition of M315 Synthesis by Id^{315}-specific Suppressor T Cells. Inhibition of M315 secretion could have resulted from (a) inhibition of M315 release without inhibition of M315 synthesis, (b) inhibition of transcription or translation of M315 messenger RNA (mRNA), or (c) an increased rate of intracellular degradation of M315. The first possibility was considered unlikely because continued M315 synthesis in the presence of a block in release would be expected to result in the intracellular
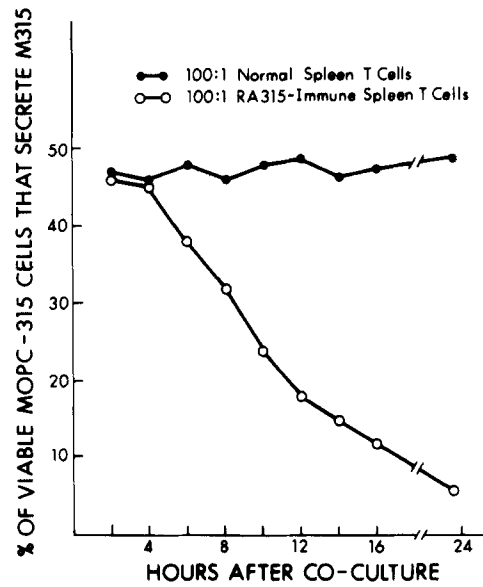
Fig. 1.   Data from a representative experiment showing the time-course of inhibition of M315 secretion from MOPC-315 cells in vitro. (O) nylon wool-purified T cells from Id^{315}-immune donors; (●) nylon wool-purified T cells from normal donors. See Material and Methods for details.

TABLE V

T Cell Suppression of M315 Synthesis and Secretion

Effector cells added to MOPC-315 cells	[^{3}H]Leucine incorporated into M315*	
	Supernatants‡	Intracellular§
None	34,119 ± 2,539‖	144,868 ± 2,319
100 × normal spleen T cells	31,919 ± 3,630	108,329 ± 1,705
100 × RA315-immune spleen T cells	1,285 ± 245	2,932 ± 1,212

* Effector and tumor cells were co-cultured for 24 h before a 24-h pulse of [^{3}H]leucine, specific immuno-precipitation of M315 was then used.
‡ 1 ml supernatant from the 48-h co-culture was immunoprecipitated for [^{3}H]leucine incorporation into secreted M315.
§ The co-cultured cells were lysed with 0.5% NP40, the subcellular organelles removed by centrifugation, and the supernatant immunoprecipitated.
‖ Mean cpm ± 1 SEM for three experiments.

accumulation of large masses of cytoplasmic M315. In a previous study, tunicamycin was shown to inhibit secretion but not synthesis of M315 (16), and this was accompanied by a striking dilatation of endoplasmic cisternae filled with M315. In the present studies, inhibition of M315 secretion by suppressor T cells was not accompanied by ultrastructural changes in the MOPC-315 cells (data not shown).

To evaluate changes in M315 synthesis, we measured incorporation of [^{3}H]leucine into secreted and intracellular M315. We observed a >90% inhibition of incorporation into both intracellular and secreted M315 in the presence of Id^{315}-specific suppressor T cells (Table V).

To determine whether inhibition of M315 synthesis was selective, studies were performed in a Marbrook apparatus so that the total protein synthesis by MOPC-315

Table VI

*Diffusable Product Selectively Inhibits M315 Synthesis**

MOPC-315 cells added to upper Marbrook chamber with lower chamber containing	[³H]Leucine incorporation into MOPC-315	
	M315‡	TCA-precipitated protein§
Media	124,936 ± 5,158‖	236,639 ± 6,041
200 × normal spleen T cells	107,254 ± 2,593	210,733 ± 6,338
200 × RA315 immune spleen T cells	9,092 ± 1,510	112,213 ± 4,481

* Effector and tumor cells were co-cultured in Marbrook culture system for 48 h before 24-h pulse of [³H]leucine.
‡ Specific immunoprecipitation of M315 was used.
§ 5% cold TCA precipitation.
‖ Mean cpm ± 1 SEM for three experiments.

cells could be measured. It was possible to use the Marbrook apparatus because in pilot studies (data not shown) we demonstrated that the Id^{315}-specific T cells mediated the suppression of M315 secretion across the 0.22-μm pore membrane of the Marbrook chamber. [³H]leucine incorporation into intracellular M315 and the TCA-precipitable fraction of MOPC-315 cells showed that the inhibition of protein synthesis was selective for M315 (Table VI). When suppressor T cells and MOPC-315 cells were incubated in separate compartments of the Marbrook chambers for 48 h and were then pulsed with [³H]leucine for 24 h, we observed an ~50% decrease in total protein synthesis in MOPC-315 cells, and the entire decrement could be accounted for by the decrease in M315 synthesis (Table VI). These findings strongly suggest that inhibition of M315 secretion by the suppressor T cell product is achieved by a selective down-regulation of M315 synthesis. Although the inhibition of secretion by increased intracellular degradation of M315 has not been formally ruled out, this possibility appears highly unlikely for two reasons: (a) because non-M315 protein synthesis was not influenced by the suppressor T cells, intracellular catabolism of M315 would have to be highly selective; (b) when secretion but not synthesis of M315 was blocked by tunicamycin (16) no evidence for intracellular degradation of M315 was detected. We established that inhibition of M315 biosynthesis was reversible (data not shown). When suppressed MOPC-315 cells were removed from the Marbrook chambers and cultured in the absence of T cells for several days, the initial frequency of M315-secreting cells was reestablished. Furthermore, the inhibition could also be quickly reversed by a brief treatment of the suppressed MOPC-315 cells with pronase, before plaquing, corroborating earlier in vivo results (9, 10).

Discussion

The studies presented here represent extensions of previous in vivo findings and have begun to establish the mechanism of inhibition of M315 secretion from MOPC-315 cells that is mediated by Id^{315}-specific suppressor T cells. These studies have established that inhibition of M315 secretion is mediated by a Lyt-2^{+} cell that expresses a surface membrane receptor for Id^{315}. The suppressor signal is a diffusable product that acts directly on M315-secreting myeloma cells. Inhibition of M315 secretion is T cell dose-dependent, Id^{315}-specific, reversible, and results from inhibition

of M315 synthesis. The inhibition of protein synthesis in the myeloma cell is selective for the myeloma protein.

The precise molecular mechanism of selective inhibition of M315 synthesis is yet to be established. It is highly unlikely that engagement per se of surface membrane Id^{315} by an anti-Id^{315} product of the T cell could account for suppression of synthesis. The engagement of surface membrane Id^{315} on MOPC-315 cells by a-Id^{315} antibodies results in a clearance of surface Id^{315} without any effect on M315 secretion.[2] Furthermore, engagement of surface Id^{315} by TNP-carrier in the presence of carrier-specific helper T cells results in an increase of M315 secretion (17). These findings (and others reviewed in reference 7) support the view that surface membrane Id^{315} functions as a focusing device for a multiplicity of immunoregulatory effectors and that the quality and intensity of the regulatory effect observed is probably dictated by the effector that is focused and not simply by engagement of the membrane Id^{315} per se.

It is likely that the selective inhibition of M315 synthesis is achieved at the level of transcription or translation. Translational control could involve mRNA processing or degradation, but would have to be highly selective to account for our findings. Control at the level of transcription is more likely and is consistent with the kinetics of inhibition reported here. The time-course of inhibition (Fig. 1) is similar to that reported for the half-life of mRNA for both heavy and light immunoglobulin chains (18). Studies in progress using Northern blot analysis of suppressed MOPC-315 cells might help clarify the precise mechanism of this control. An interesting aspect in this regard is the persistent expression of surface membrane M315 in cells in which secretory M315 is suppressed. Although the maintenance of membrane M315 might be accounted for by a long turnover time or a large intracellular pool of membrane M315, it could also reflect separate regulation of the biosynthetic pathways for membrane and secretory M315.

The experiments using Marbrook chambers establish that a diffusable effector acts on the target myeloma cell. Although the present studies have not distinguished between a product produced by the a-Id^{315} T cell or by another T cell in the immune cell sample, preliminary results using an a-Id^{315}-specific T cell hybridoma indicate that the effector is a product of the a-Id^{315} T cell (G. L. Milburn and R. G. Lynch, unpublished observations). The present studies clearly establish that suppressor T cells can act directly on B cells as late in B cell development as the actual antibody-secreting cell. These suppressor T cells do not work indirectly by suppressing a helper T cell and they do not require an adherent acessory cell. Several laboratories have presented data suggesting that suppressor T cells can act directly on nonneoplastic antibody-secreting cells (19–22).

Finally, hapten-specific myelomas and other neoplastic lymphoid clones (23, 24) provide unique opportunities to establish the molecular and cellular mechanisms of B cell regulation. An elegant example of their utility is the recent study of Abbas et al. (25), in which myeloma-myeloma hybrids were used to analyze immunoglobulin regulation. Coupled with recombinant DNA technology, the study of cloned B cells might lead to an understanding of B cell regulation in precise molecular terms.

Summary

In previous studies, BALB/c mice immunized with the trinitrophenyl-specific IgA protein (M315) produced by MOPC-315 developed idiotype (Id^{315})-specific T cells

that suppressed M315 secretion in vivo. In the present in vitro studies, we show that inhibition of M315 secretion is mediated by a θ^+, Lyt-1$^-$2$^+$ cell that expresses a surface membrane receptor for Id^{315}. The suppressor signal is a diffusable product that acts directly on M315-secreting myeloma cells. Inhibition of M315 secretion is T cell dose-dependent, Id^{315}-specific, reversible, and occurs without any effect on MOPC-315 growth, viability, or surface membrane expression of M315. Inhibition of M315 secretion results from a selective inhibition of M315 synthesis in the myeloma cell. These studies provide new insight into the mechanisms of direct B cell regulation by idiotype-specific T cells.

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