SPECIFIC SUPPRESSIVE FACTORS PRODUCED BY HYBRIDOMAS DERIVED FROM THE FUSION OF ENRICHED SUPPRESSOR T CELLS AND A T LYMPHOMA CELL LINE*

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The induction of tolerance to human gamma globulin (HGG)[1] in mice was associated with the existence of suppressor T cells (1). These were antigen-specific since suppression, like help, was dependent for its expression on the inducing antigen, HGG (1), and could be abrogated specifically by pretreatment with ^{125}I-labeled anti-HGG under suicide conditions (2). Recently, we extracted an antigen-specific suppressive factor from splenic T cells of mice tolerant to HGG (3). This material did not bear immunoglobulin markers but carried a determinant coded by the I-J locus of the major histocompatibility complex. Its properties were indeed very similar to those reported by other investigators for primed T cells (4), cells from nonresponder mice (5), and allotype-specific cells (6).

The establishment of T-cell hybridomas with specific suppressor function would provide an ideal tool to characterize further the I-J bearing antigen-specific suppressor factor and its molecular mechanism of action. Suppressor T cells with defined specificity form, however, a very minor proportion of the total spleen population and the chances of fusing one such cell with a thymoma cell line must necessarily be very low. Recently, we were able to enrich for I-J$^+$ antigen-specific suppressor T cells by allowing Ig$^-$ spleen cells from tolerant mice to bind to dishes coated with HGG (7). We therefore utilized this enrichment procedure to provide a richer source of specific suppressor T cells for fusing with the T lymphoma cell line, EL-4. Since the suppressor T cells have I-J determinants on their surface (7), we used a monospecific anti-I-J serum together with a fluorescein-conjugated rabbit anti-mouse immunoglobulin (MIg) reagent to select through a fluorescent activated cell sorter (FACS), after the fusion process, those normal and hybridoma cells with I-J on their surface. The separated cells were then cultured and cloned, and examined for surface markers, karyotype, and suppressive function. The results are given here and

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[1] *Abbreviations used in this paper:* DME, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DNP, dinitrophenyl; FITC, fluorescein isothiocyanate; Fla, flagellin of *Salmonella adelaide*; HGG, human gamma globulin; MIg, mouse immunoglobulin; PEG, polyethylene glycol; PFC, plaque-forming cells.

show that hybridoma lines with specific suppressive properties can be established.

Materials and Methods

Mice. Male and female mice of the highly inbred CBA/H/Wehi were obtained from the specific pathogen-free breeding colony of the Walter and Eliza Hall Institute, Melbourne. They were used at ages 8- to 10-wk-old.

Antigens. HGG (Cohn Fraction II) was purchased from the Commonwealth Serum Laboratories, Melbourne, as a solution containing 160 mg/ml. Deaggregated HGG was prepared according to the method of Basten et al. (1). Polymerized flagellin (Fla) of *Salmonella adelaide* was kindly supplied by Dr. J. Pye of the Walter and Eliza Hall Institute. The hapten dinitrophenyl (DNP) was purchased from Eastman Kodak, Rochester, N. Y. in the form of sodium sulfonate salt. It was coupled to protein carriers as described by Eisen (8). The following substitution ratios were used: DNP_8HGG and DNP_6Fla. Horse erythrocytes were obtained from the Commonwealth Serum Laboratories and stored in Alsever's solution.

Immunization. Mice were primed by intraperitoneal injections as follows: 400 μg alum precipitated HGG together with 2×10^9 killed *Bordetella pertussis* organisms (Commonwealth Serum Laboratories, Melbourne); 20 μg of DNP-Fla. Alum precipitation was carried out as described previously (9). Spleen cells from primed mice were generally used 4- to 8-wk postpriming.

Tolerance Induction. HGG was dialyzed overnight with saline and centrifuged to obtain deaggregated protein as described by Basten et al. (1). 2.5 mg deaggregated HGG was injected intravenously into mice and the spleens of these mice were used 15 days later.

Antisera. Anti-Thy-1.2, anti-H-2^k (C57BL/6 anti-CBA), anti-H-2^b (CBA anti-C57BL) (9), anti-I-J^k (B10.A(3R) anti-B10.A(5R)), anti-I-J^b, sera, and fluorescent rabbit anti-MIg serum were used. The anti-I-J^k serum was kindly supplied by Dr. Tomio Tada, Chiba University, Japan. The rabbit anti-MIg antibody labeled with fluorescein isothiocyanate (FITC) was kindly obtained from James Goding (Walter and Eliza Hall Institute). The antisera were extensively absorbed with normal mouse thymocytes and/or three times with an equal volume of tumor cells at 0°C for 30 min. The absorbed reagents were found not to react with tumor cells by membrane fluorescence. Anti-I-J^k serum absorbed with cells from hybridoma lines that contained I-J^{k+} cells no longer stained cells in suspensions of tolerant spleen cells enriched for suppressor T cells.

Preparation of Antigen-Specific Suppressor T Cells. The method used for the preparation and enrichment of antigen-specific suppressor T cells was described previously (7). In brief, 4×10^7 spleen cells from mice tolerized with 2.5 mg of deaggregated HGG 15 days previously, were added to anti-MIg antibody-coated Petri dishes to remove Ig^+ cells. After incubation at room temperature for 1 h, nonadherent cells (T cells) were removed and incubated in HGG-coated dishes under the same conditions. Nonantigen binding cells were discarded and the plates were then placed on ice for 15 min. The antigen-binding cells could easily be released by pipetting cold medium. This population was used for fusion with the T lymphoma cell lines, EL-4 or L5178.

T Lymphoma Cell Lines. Two T-cell lines were used in cell fusion experiments. The EL4 line was kindly supplied by Dr. Cerottini in 1971 and has been passaged here since that time in cell culture. It originated from a chemical carcinogen-induced lymphoma in a C57BL/6 mouse (10) and is Thy-1.2 and H-2^b positive. The line L5178 was kindly supplied by Dr. Boyle in 1977. It was originally derived from a population of L5178Y that had been treated with the mutagen ICR-372 (11). The parent strain originated from a leukemic DBA/2 mouse (12). The mutant cells, which lack hypoxanthine/guanine phosphoribosyl transferase, cannot proliferate in hypoxanthine aminopterin thymidine medium (11). They express Thy-1.2 and the H-2^d alloantigens 4, 8, and 35.

Cell Hybridization. A mixture of 5×10^6 enriched suppressor T cells and $5\text{–}10 \times 10^6$ T lymphoma cells in Dulbecco's modified Eagle's medium (DME) was centrifuged at 400 *g*. The medium was removed and to the pellet was added 2 ml of polyethylene glycol (PEG) dimethylsulfoxide solution (reagent A: 1 weight of PEG, mol wt 4,000, plus 1.4 vol of 15% dimethylsulfoxide [DMSO] in DME). This was gently mixed with a broadened pipette. Immediately after this step, the mixture was transferred into 2 ml of 50% (wt/vol) PEG (reagent B: 1 weight of PEG plus 1 vol of DME) and mixed well as above. The suspension was then gradually diluted to 16 ml of serum-free DME and further diluted with 180 ml of DME containing 13% fetal calf serum (FCS). Each of

the above procedures was completed within 1 min. The mixture was then incubated at 37°C in 5% CO_2 in air. 3 h after incubation the cells were washed four times with medium and cultured for 1 to 2 days in 10% FSC-DME at 37°C.

Separation of Fused I-J$^+$ Cells. 1–2 days after the cell fusion, cells were harvested and the cell membrane was stained by a monospecific anti-I-J serum and an FITC-conjugated rabbit anti-MIg antibody. The stained cells were washed four times with Hepes-buffered DME (containing 20 mM Hepes). The I-J$^+$ cells were then separated by a fluorescence activated cell sorter (FACS-II, Becton-Dickinson Electronics Laboratory, Mountain View, Calif.) and cultured at 37°C in 5% CO_2 in air in 0.3% agar (Difco Laboratories, Detroit, Mich.) containing 5% FCS-DME. After 12 days, colonies of fused cells were transferred and cultured in 10% FCS-DME to characterize their surface markers, chromosomes, and suppressive activities. This was repeated at subsequent-intervals.

Characterization of Surface Markers. The surface markers of fused cells were characterized by immunofluorescence and by rosetting. For immunofluorescence, fused cells were exposed to specific antisera at room temperature for 30 min with occasional shaking and then washed four times with Hepes-DME. Cells were further stained with FITC-conjugated rabbit anti-MIg serum at a dilution 1:10 under the same conditions as above. 30 min later, the cells were washed four times with medium, smeared on glass slides, and checked under the fluorescence microscope. The technique of Sandrin et al. was used for marker detection by rosetting (13).

Chromosome Cytology. For chromosome studies cultured cells were spread by an air drying technique (14).

Assay for Suppressive Activity of Extract from Fused Cells on the in Vivo Adoptive Secondary Anti-Hapten Antibody Response. The cells derived from a single colony of fused cells separated by FACS were sonicated as described previously (3). The cell-free extract was tested in vivo on the secondary adoptive response of irradiated mice given 5×10^6 HGG-primed and 5×10^6 DNP-Fla-primed spleen cell intravenously, 10^8 HRBC intravenously, and 100 μg DNP-HGG intraperitoneally. Extract equivalent to 10^6 viable hybridoma cells was injected intravenously at the time of cell transfer and 7 days later the anti-DNP response was measured.

Irradiation. Mice to be irradiated were exposed to 800 rads total body irradiation as described in detail elsewhere (9). Cell suspensions were injected into irradiated mice within 3 h of exposure.

Detection of Antibody-Forming Cells. Both direct and indirect plaque-forming cells (PFC) were detected by techniques described elsewhere (9). For measurement of anti-DNP PFC, DNP sulfonate was coupled to the $F(ab')_2$ fragment of rabbit IgG anti-sheep erythrocyte as described previously (8), and the DNP-$F(ab')_2$ was then used for sensitization of sheep erythrocytes.

Statistical Analyses. These were performed as described before (9).

Results

Separation of I-J^{k+} Hybrid Cells by FACS. The antigen-specific CBA (H-2^k) suppressor T cells, enriched by the antigen-coated Petri dishes, were fused with C57BL/6 (H-2^b) EL-4 tumor cells. 1 day after cell hybridization, the cells were reacted with anti-I-J^k serum or normal C57BL/6 serum, and stained with FITC-conjugated rabbit anti-MIg serum as described in Materials and Methods. The fluorescence distribution of cells was then analyzed with the FACS II. Fig. 1 shows the fluorescence profile of cells labeled with the reagents. The staining with anti-I-J^k serum (thick line) provided a broad bright peak and computer analysis of this indicated that about 5% of the total population was stained by anti-I-J^k serum. On the other hand, cells stained with normal C57BL serum and FITC-conjugated rabbit anti-MIg (thin line) showed only a dull peak, judged to be nonspecific since no significant fluorescence could be detected when hybridoma cells were reacted only with FITC-conjugated rabbit anti-MIg. The fluorescent I-J^{k+} cells were sorted out by the FACS and seeded into 0.3% agar plates with 5% FCS-DME. There were about 10 colonies per agar plate 10–12 days after hybridization; single colonies were picked up by a pipette under the

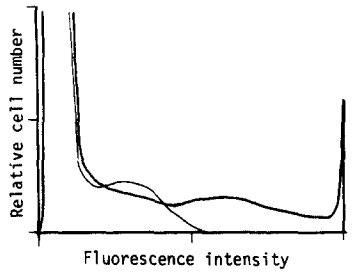

FIG. 1. Fluorescence distribution of hybridoma cells, labeled with anti-I-J^k serum and FITC-conjugated rabbit anti-MIg. 1 day after hybridization of enriched suppressor CBA (H-2^k) T cells with the tumor cell line EL-4 (H-2^b), cells were incubated for 30 min at room temperature with the monospecific anti-I-J^k serum, washed four times, and incubated for an additional 30 min at 37°C with FITC-conjugated rabbit anti-MIg. The cells were then washed and analyzed by the FACS. Distributions are based on cumulative analyses of 40,000 viable cells. The thick line indicates the fluorescence profile of cells incubated with a 1:8 dilution of anti-I-J^k serum. The thin line indicates the pattern of cells stained with a 1:10 dilution of normal mouse serum.

microscope and cultured in 10% FCS-DME solution for further characterization.

Direct Demonstration of the H-2^k Gene Products on Hybridoma Cells by Membrane Fluorescence. The cells from each colony were further investigated for surface markers by using various antisera absorbed appropriately. More than 95% of cells from either EL-4 or any of the hybridoma cell lines failed to show detectable fluorescence after direct exposure to FITC-conjugated rabbit anti-MIg. About 60–90% of the cells in each hybridoma clone, but never 100%, were stained by FITC-conjugated rabbit anti-MIg after preincubation with anti-H-2^b, anti-H-2^k, anti I-J^k, and anti-Thy-1.2 sera. No fluorescence was obtained by staining EL-4 tumor cells with anti-H-2^k and anti-I-J^k sera. I-J^b was not detectable on hybridoma cells nor on EL-4.

Progressive Loss of H-2^k Gene Products from Hybridomas. Cell surface markers on lines found to have specific suppressive activity (see below) were determined at intervals after cell fusion. The situation is summarized in Table I. The data clearly show loss of H-2^k gene products (both I-J^k and H-2.23) by 3 mo after cell fusion. A number of other cell lines (not all shown in Table) also showed the same phenomenon.

Karyotype of Hybridoma Cell Lines. Soon after cell fusion most hybridoma cells had between 60 and 90 chromosomes per cell, but this number rapidly dropped in the established clones to near 40 within 3 mo. Table II gives typical chromosome numbers for cells of a parent hybridoma line (22) examined 4 wk after cell fusion. From this line were derived by cloning lines 72, 73, 119, and 125. Initially the majority of cells from these lines were near-tetraploid but became near-diploid 3 mo after cell fusion. One cell line (77) appeared to contain two cell populations: the majority were diploid but a minority population was near-tetraploid (Table II).

Suppression of the in Vivo Adoptive Anti-DNP PFC Response by Cell-Free Extracts from Hybridoma Cell Lines. Previous work showed that an extract obtained by sonication of spleen cells from mice tolerant to HGG could specifically suppress the adoptive DNP PFC response in irradiated recipients of HGG-primed and DNP-primed spleen cells given DNP-HGG. The active mate-

TABLE I

Loss of H-2^k Gene Products from Hybridoma Cell Lines with Time after Cell Fusion

Cells	Time after fusion	Percent of cells with following markers				
		Ig	H-2.23*	H-2.33*	I-J^k	Thy-1.2
Normal CBA spleen (Ig$^-$ cells)	–	<1	82	<5	<5	73
Normal C57BL spleen (Ig$^-$ cells)	–	<1	<5	>90	<5	81
EL-4	–	<1	<5	76	<5	81
Line 72	6 wk	<1	45	ND	40	>90
Line 73	6 wk	<1	20	ND	20	>90
Line 77	6 wk	<1	80	ND	>90	>90
Line 104	6 wk	<1	80	ND	>90	>90
Line 110	6 wk	<1	70	ND	80	>90
Line 111	6 wk	<1	ND	ND	30	>90
Line 119	6 wk	<1	80	ND	80	>90
Line 125	6 wk	<1	80	ND	70	>90
Line 72	>3 mo	<1	<5	58	<5	>90
Line 73	>3 mo	<1	<5	66	<5	>90
Line 77	>3 mo	<1	16	45	10	>90
Line 104	>3 mo	<1	<5	47	<5	>90
Line 110	>3 mo	<1	<5	55	<5	>90
Line 111	>3 mo	<1	<5	55	5	>90
Line 119	>3 mo	<1	<5	50	<5	80
Line 125	>3 mo	<1	20	61	15	85

ND = not done.

* 4 wk after fusion, >90% of cells of the parent line which gave rise to the lines listed above had both H-2.23 and H-2.33 antigens.

rial was shown to carry a determinant coded by the I-J locus of the major histocompatibility complex (3). It was thus of interest to determine whether sonicated material from hybridoma cell lines bearing the I-J^k determinant would be specifically suppressive in the in vivo model. Extracted material corresponding to 10^6 viable cells was injected into heavily irradiated recipients of 5×10^6 DNP-primed and 5×10^6 HGG-primed spleen cells, 100 μg DNP-HGG and 10^8 HRBC. 7 days later, direct and indirect anti-DNP and anti-HRBC PFC per spleen were determined. The results obtained in a single experiment with 18 hybridoma cell lines which contained I-J^{k+} cells are shown in Table III. Three different groups can be identified. Lines 72, 73, 77, 111, 119, and 125 contained from 20 to >90% I-J^{k+} cells; extracts from these lines produced significant specific suppression ranging from 61 to 80%. Lines 106, 109, 112, 121, and 127 had from 10 to >90% I-J^{k+} cells and extracts from these lines produced nonspecific suppression ranging from 63 to 99%. Finally, lines 75, 102, 114, 117, 118, 122, and 128 produced no suppression even though their content of I-J^{k+} cells ranged from 20 to >90%. Specific suppression, when it occurred, generally affected the indirect DNP response more markedly than the direct.

Specific suppressive activity obtained with material from lines 72, 73, 111, and 119 was no longer evident 3 mo after cell fusion (Table IV). This loss parallels that observed for H-2^k gene products (Table I) and the change in karyotype toward the diploid state (Table II). Sonicates from line 77 still had some specific suppressive activity 6 mo after cell fusion (Table IV).

TABLE II
Karyotype of Hybridoma Cells

Cell lines*	Weeks after fusion	Number of metaphases with following chromosome number								
		30–35	36–39	40	41–50	51–60	61–70	71–80	81–90	>90
EL-4	–	0	3	11	2	0	0	0	0	0
22	4	0	0	0	1	3	7	16	6	2
72	14	1	7	7	24	0	0	0	0	0
73	14	0	3	17	9	1	0	0	0	0
77	24	0	4	18	7	1	0	5	3	0
119	14	0	18	6	6	0	1	0	0	0
125	14	0	9	19	9	2	1	0	0	0

* Lines 72, 73, 119, and 125 were originally derived from clones established from parent line 22 and were initially near-tetraploid.

Discussion

Several investigators have recently reported successful fusion of T cells and thymoma cell lines. When normal mouse spleen cells were used as a source of T cells, the hybridomas expressed only T-cell markers suggesting preferential hybridization of thymoma cells with T cells or extinction of B-cell marker expression in the hybrids (15, 16). None of the T-cell hybridomas, so far studied and reported in the literature to date, have expressed normal T-cell functions (16, 17). The present report thus constitutes the first documented investigation demonstrating the production of T-cell hybrids with specific T-cell function. The fusion of antigen-specific, I-J$^+$, suppressor T cells and the T lymphoma cell line, EL-4, was facilitated considerably by the use of two procedures: the method we developed to enrich antigen-specific, I-J$^+$, Ig$^-$ cells from tolerant spleen (7) and the selection, subsequent to fusion, of I-J$^+$ cells by appropriate fluorescent reagents and the FACS. The lines established after FACS separation were shown to be hybridomas of CBA T cells (H-2^k) and EL-4 (H-2^b), since they carried no Ig determinants but expressed both H-2^k (H-2.23) and H-2^b (H-2.33), and also I-J^k, surface markers. In addition, the karyotype of the majority of the cells observed soon after fusion showed a chromosome number considerably greater than diploid, often near tetraploid.

In vivo assays for suppression by sonicates of hybridoma cells enabled the lines to be classified into three groups with different activities: antigen-specific, nonantigen-specific, and not suppressive. It is noteworthy that the material from lines with specific activities produced mild or no suppression of the direct DNP PFC response but markedly suppressed the indirect antibody response. A similar finding was reported with extracts of HGG-tolerant spleen cells (3). The activity of the lines could not be predicted from their content of I-J^{k+} cells: thus, some lines with >90% I-J bearing cells had no suppressive activity (e.g. line 75), whereas others with only 20% such cells were specifically suppressive (line 73). It is not clear whether the I-J gene product is expressed solely on cells with suppressive activities or whether it can be expressed also on cells with other activities. Neither is it known whether cell surface expression of the I-J determinant varies with the cell cycle. Further studies of I-J^{k+} hybridoma cell lines with suppressive properties may elucidate these questions. Other expla-

TABLE III

*Effect of Cell-Free Extracts from I-J^{k+} Hybridoma Cell Lines on the in Vivo Adoptive Anti-DNP PFC Response**

Line number	I-J^k bearing cells‡	Indirect anti-HRBC PFC per spleen at 7 days	Anti-DNP PFC per spleen at 7 days		Specific or nonspecific suppression
			Direct	Indirect	
	%				%
None		6,450 (1.18)§	7,030 (1.07)	104,370 (1.14)	
EL-4	<5	5,450 (1.22)	5,440 (1.34)	95,860 (1.08)	8, Nonspecific
72	30	7,550 (1.40)	15,140 (1.19)	37,940 (1.12)	64, Specific
73	20	9,730 (1.30)	10,820 (1.23)	21,340 (1.40)	80, Specific
75	>90	2,180 (1.58)	15,450 (1.16)	132,080 (1.11)	0
77	>90	4,970 (1.31)	3,470 (1.30)	33,980 (1.13)	67, Specific
102	50	2,300 (1.16)	8,130 (1.24)	109,380 (1.28)	0
106	50	3,040 (1.50)	4,420 (1.39)	37,170 (1.31)	64, Nonspecific
109	10	1,040 (1.70)	3,950 (1.55)	38,670 (1.32)	63, Nonspecific
111	30	5,630 (1.45)	5,930 (1.23)	34,280 (1.19)	67, Specific
112	>90	480 (2.36)	1,000 (1.68)	13,550 (2.26)	87, Nonspecific
114	20	9,610 (2.01)	14,000 (1.34)	116,210 (1.33)	0
117	30	3,360 (1.17)	14,540 (1.30)	152,770 (1.12)	0
118	>90	6,640 (1.24)	14,190 (1.23)	105,640 (1.42)	0
119	80	7,490 (1.18)	1,860 (1.18)	28,230 (1.14)	73, Specific
121	90	120 (3.52)	2,970 (1.58)	18,460 (1.97)	82, Nonspecific
122	90	6,830 (1.73)	13,750 (1.14)	138,260 (1.09)	0
125	70	6,510 (1.36)	6,050 (1.23)	40,310 (1.29)	61, Specific
127	>90	1,210 (1.22)	70 (1.44)	1,430 (1.32)	99, Nonspecific
128	80	5,400 (2.32)	14,190 (1.23)	105,640 (1.47)	0

* Irradiated mice received extract equivalent to 10^6 cells, 5×10^6 HGG primed spleen cells, 5×10^6 DNP-Fla primed spleen cells, 10^8 HRBC intravenously, and 100 μg DNP-HGG intraperitoneally.

‡ Determined by immunofluorescence.

§ Geometric means and SE. Five to six mice per group.

nations for the inability of the anti-I-J^k serum to stain 100% of cells in some of the recently established hybridoma clones include possible contamination of the clones with the parent EL-4 cells and loss of chromosomes occurring in the hybridomas soon after cell fusion.

Various cloned hybridoma lines were investigated for surface markers, karyotype, and suppressive activity at progressively greater intervals after cell fusion. Most lines selectively lost the characteristic properties derived from the normal CBA parent. Thus, some 3 mo after cell fusion, only <5 or 10% of cells bearing H-2^k or I-J^k gene products could be detected. Likewise, no significant suppression was observed with extracts of cells of four out of five hybridomas from which specifically suppressive material had been obtained several weeks before. In parallel with this loss of surface markers and suppressive function was a change in the karyotype from near-tetraploid to near-diploid. A similar loss in chromosomes has been reported in T-cell hybridomas by Hammerling (16).

The most difficult situation to be encountered with T-cell hybridomas may not be the successful fusion of a thymoma cell with a normal T cell that has a specific function, but the progressive loss, during the growth of the hybridomas,

TABLE IV
*Loss of Specific Suppressive Effect of Cell-Free Extracts from I-J^k + Hybridoma Cell Lines with Time after Cell Fusion**

Line number	Percent suppression of DNP response at following times after cell fusion		
	6 wk	10 wk	>3 mo
72	64	24	0
73	80	14	0
77	67	59	46
111	67	65	0
119	73	66	0
125	61	ND	ND

ND, not done.
* Irradiated mice received cells and antigen as mentioned in a footnote to Table III.

of those properties derived from the normal parent cell. Conceivably, this may result from extinction by the tumor genome of the expression of the gene products of the normal cell. Alternatively, as seems to have been the case here, there may be a selective loss of chromosomes derived from the normal parent. It is possible that the state of differentiation of the tumor line could determine the subsequent behavior of the hybridoma—the extent to which the normal effector T cell function may be preserved. Preliminary results we have obtained with hybridomas produced by the fusion of enriched suppressor T cells and the HAT-sensitive thymoma cell line, L5178, indicate that some of the hybrids still express H-2^k and I-J^k antigens 18 wk after cell fusion. It would therefore seem profitable to utilize a variety of T lymphoma cell lines for hybridization so as to determine which line may be optimal for the study of a particular effector T-cell function.

Summary

A cell fusion technique was used to produce hybridomas between the T lymphoma cell line, EL-4, derived from C57BL (H-2^b), and an enriched population of human gamma globulin (HGG)-specific suppressor T cells prepared from the spleens of HGG-tolerant CBA mice (H-2^k). Membrane fluorescence analysis of the hybridoma cells within 6 wk of cell fusion revealed expression of H-2^k and I-J^k gene products as well as H-2^b antigens. Sonicates prepared from hybridomas which contained I-J^k cells were tested for suppressive activity in vivo in irradiated mice given HGG-primed cells, dinitrophenyl (DNP)-primed cells, HGG-DNP, and horse erythrocytes. Among 18 such hybridoma lines, 6 showed specific suppressive activity, 5 nonspecific suppression, and 7 no suppression. Most lines progressively lost, with time, those properties derived from the normal parent cell. By about 3 mo after fusion few cells expressed CBA markers and only one cell line (number 77) retained some specific suppressive activity. In parallel with the losses was an alteration in chromosome number from near-tetraploid, soon after cell fusion, to near-diploid. Preliminary results with the T lymphoma-sensitive hypoxanthine aminopterin thymidine cell line, L5178, indicate retention of the expression of surface markers derived from the normal

parent for 18 wk after hybidization. This suggests that T lymphoma cell lines may have to be screened for their capacity to produce hybridomas with stable properties.

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