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In Vitro Suppression of T-Cell Mitogenic Response and Tumor Cell Proliferation by Spleen Macrophages from Normal Chickens

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Adherent cells isolated from spleen of normal specific pathogen-free chickens inhibited mitogen-induced blastogenesis of autochthonous, syngeneic, or allogeneic lymphocytes. The adherent cells were also inhibitory to in vitro proliferation of cells of a rapidly dividing tumor line, MDCC-MSB-1, derived from a lymphoma induced by Marek's disease virus. The effector cell of suppression of both lymphoproliferative functions appeared to be a macrophage because the suppressive activity of adherent cells could be abrogated by pretreatment with carrageenan but not with antisera specific to chicken T or B cells. The proportion of macrophages needed for effective suppression was substantially higher than the proportion of macrophages ordinarily present in spleen of normal, unstimulated chickens. This heretofore unrecognized suppressive capability of normal, presumably resting macrophages in chickens needs to be evaluated in light of the fact that suppressor macrophages have been detected in certain infections.

Macrophages regulate many aspects of the immune system. For instance, they enhance in vivo antibody response to antigens (17, 28) and are essential for in vitro antibody synthesis against certain antigens (29). Macrophages seem particularly involved in early activation of immune cells by stimulants in vitro; e.g., they are essential for proliferative responses of lymphocytes to soluble and allogeneic antigens (12, 35, 49) and possibly also to mitogens (22, 23). Generation of cytotoxic T lymphocytes and production of lymphokines by immune cells may also require participation by macrophages (19, 41, 45, 47, 48).

In contrast to the important role that macrophages may play in generating or enhancing immune responses, these cells under certain circumstances can also be potent suppressors of immune reactivity. Some evidence shows that macrophages from normal animals (8, 33), animals inoculated with reticuloendothelial system stimulants (16, 36, 37), or animals carrying tumor burden (10, 15) may inhibit certain lymphocyte-mediated immune responses. Signals that regulate enhancing or suppressive functions of macrophages in in vivo immune responses to tumor or nontumor antigens are not clearly defined; thus, there is need for further studies on role of macrophages in host immunity.

The results presented in this study show that normal specific-pathogen-free chickens contain cells adherent to plastic, presumably macrophages, that were strongly inhibitory to two in vitro lymphoproliferative responses, namely, mitogen-induced blastogenesis and tumor cell proliferation. The presence of suppressor macrophages in normal chickens has not been recognized previously, although Lee et al. (21) in our laboratory recently reported that spleen cells (SC) from chickens infected with Marek's disease virus or herpesvirus of turkeys were inhibitory to mitogenic response of normal lymphocytes and that this inhibition was mediated by macrophages present in spleens of virus-infected chickens.

MATERIALS AND METHODS

Chickens. SC donor chickens were F_1 progeny of a cross between lines 151_5 and 7_1 (15 × 7). In experiments in which allogeneic restriction of suppressor cells was examined, lines P and N chickens were also used. Chickens of lines 15 × 7, P, and N differed for major histocompatibility B-haplotypes. All chickens were maintained in this laboratory under specific pathogen-free conditions and were 4 to 10 weeks old at the time of use. The chickens lacked detectable evidence of infection with common poultry pathogens, including Marek's disease virus or herpesvirus of turkeys.

Tumor cell line. The lymphoblastoid cell line MDCC-MSB-1, derived from a Marek's disease lymphoma, was obtained from S. Kato (Osaka University, Osaka, Japan) (1). A stock of MDCC-MSB-1 cells suspended in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) containing 10% fetal calf serum (FCS) and 10% dimethyl sulfoxide was frozen at −196°C (30). Cells from this stock within 15 to 20 further serial passages were used for this study. The tumor cells were propagated as suspension cultures in large petri

plates and were routinely used within 24 h of subculture. The cells were usually >95% viable when used. Cell viability was tested by trypan blue exclusion.

Spleen cell suspensions. Donor chickens were killed by CO_2 inhalation, and spleens were removed aseptically. Single-cell suspensions were prepared by the procedures detailed previously (40) with modifications. SC released into 1.1× Dulbecco phosphate-buffered saline containing 2% FCS (DPBS) were clarified on a Ficoll-Hypaque gradient at a specific gravity of 1.09 (42). Cells removed from the interphase were washed three times with DPBS before use.

Mitogenic assay. The general technique of mitogenic assay has been described (21). The test was conducted in Microtest II plates with 96 flat wells (Falcon Plastics, Los Angeles, Calif.). The mitogenic assays were set up with six wells per responder cell, and to each of three wells was added 100 μg of phytohemagglutinin-P (PHA, Difco). Each well contained 10^6 responder cells (usually SC) alone or with appropriate numbers of inhibiting cells in a total volume of 0.2 ml of RPMI 1640–5% FCS. Tests to establish optimum conditions for mitogenic stimulation revealed that SC concentrations of 0.5×10^6 to 2.0×10^6 per microwell gave a linear response and that optimum concentration of PHA per culture was 50 to 200 μg (21). The cultures were incubated for 48 h at 41°C in a humidified incubator containing 2.5 to 3.0% forced CO_2. Then 0.05 μCi of ^{125}iododeoxyuridine (0.9 to 1.1 Ci/mmol, Amersham/Searle, Arlington Heights, Ill.) was added to each well for an additional 16 h. Cells were assayed for incorporation of radioactivity by harvesting onto glass filter papers (Reeve-Angel, Chifton, N.J.) with a MASH II automatic sample harvestor (Microbiological Associates). Filters were counted for radioactivity in a Beckman 300 γ-irradiation counter (Beckman Instruments, Inc., Irvine, Calif.). The counts per minute were averaged for mitogen-containing and mitogen-lacking cultures.

Tumor cell proliferation assay. This test was set up in triplicate in Microtest II plates as described previously (21). Each well contained 0.2 ml of medium (RPMI 1640–10% FCS) with 10^4 MDCC-MSB-1 cells alone or in combination with appropriate concentrations of inhibiting cells. The cultures were incubated at 41°C in a humidified CO_2 incubator, and assays were ended at 48 h after a 4-h pulse with 0.05 μCi of [^{125}I]iododeoxyuridine. The percentage of inhibition of tumor cell proliferation was calculated as follows: percent inhibition = [(counts per minute in tumor cells alone − counts per minute in tumor cells and inhibiting cell mixture)/counts per minute in tumor cells alone] × 100.

Cell separation procedures. SC were depleted of phagocytic and adherent cells by serially treating them with carbonyl iron and passing nonphagocytic cells through rayon columns. From 2×10^8 to 3×10^8 SC suspended in 15 ml of RPMI 1640–50% FCS were mixed with 22 mg of carbonyl iron (1-1-63763, GAF Corporation, New York, N.Y.) and incubated at 37°C with continuous slow agitation. After 1 h, 15 ml of warm medium was added to the SC–iron mixture, and cells that had phagocytized iron particles were removed by passage over a strong magnet six times. The nonphagocytic cells were then passaged through a rayon column as follows. A 30-ml plastic syringe was packed with three balls of rayon cotton (Parke Davis Co., Detroit, Mich.) and sterilized by autoclaving. Columns were equilibrated with 50 ml of warm medium. Then, 2×10^8 to 3×10^8 cells in 5 ml of medium were placed on the column, and the column was incubated for 20 min at 37°C in a humidified incubator containing 2.5 to 3.0% forced CO_2. At the end of the incubation period, the nonadherent cells were eluted with 30 ml of warm medium. The macrophage-depleted SC were pelleted and used in assays. The depleted SC had <1% latex particle-ingesting cells; unfractionated SC had 3 to 4% of such cells.

Adherent cells were obtained by incubating at 37°C in 100-mm plastic petri dishes 50×10^6 SC in 10 ml of warm medium (RPMI–10% FCS). After 2 to 4 h, nonadherent cells were removed by at least five serial vigorous rinses with warm medium. Adherent cells, released by gentle scraping with a rubber policeman, were 60 to 80% viable. Plastic adherent cells derived from SC are also referred to here as macrophages despite the fact that adherent population may contain functionally and morphologically heterogeneous cells (e.g., see Table 5).

Treatment of cells with antisera to T and B cells. Antisera to T and B cells were prepared as described elsewhere (39). Rabbits were given repeated intravenous injections of viable cells from 3-week-old donor chickens. Antisera were exhaustively absorbed with heterologous (thymus or bursa) cells until nonspecific activity disappeared. The absorbed antisera were highly cytotoxic for homologous cells in a complement-dependent antibody cytotoxicity assay in which ^{51}Cr-labeled target cells were used (39). From 10×10^6 to 100×10^6 cells were reacted for 45 min at 37°C with a 1:5 final dilution of antisera and an optimum concentration of guinea pig complement (Difco Laboratories). At the end of the incubation period, the cells were washed three times with DPBS and adjusted to appropriate concentrations of viable cells.

Carrageenan treatment. Carrageenan (SEAKEM-9,RE 7059, Marine Colloids, Inc., Rockland, Maine) inactivates macrophages (2, 18). For carrageenan treatment, 10^7 cells were suspended in 5 ml of RPMI–10% FCS containing 400 μg of carrageenan per ml and incubated in plastic petri dishes for 4 h at 37°C in CO_2 atmosphere. At the end of the incubation period, adherent cells were detached with a rubber policeman and mixed with nonadherent cells. The cells were then washed three times and adjusted to an appropriate viable cell concentration before use.

Latex ingestion test. For this test, 0.3 ml of a latex 0.81 suspension (Difco Laboratories) was diluted with 3.70 ml of medium, and 0.5 ml of diluted latex suspension was mixed with 0.5 ml of cell suspension containing 2.5×10^6 cells. The mixture was placed in a 35- by 10-mm plastic petri dish. After incubation for 2 to 3 h at 41°C, adherent cells were detached with a rubber policeman and mixed with nonadherent cells. The cells were then washed three times to remove free latex particles and counted in a hemacytometer for latex-containing cells. Only the cells that had interiorized latex particles were considered positive for latex ingestion.

Immunofluorescent staining. Viable single cell

suspensions were examined for surface staining by indirect immunofluorescent test. At 4°C, 2×10^6 pelleted cells were reacted with a 1:10 dilution of rabbit antisera to chicken T or B cells. After 30 min, cells were washed with DPBS and reacted with a 1:20 dilution of fluorescein-isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (Miles Laboratories Inc., Elkhart, Ind.). After a further 30-min reaction at 4°C, cells were washed, and approximately 100 cells in each sample were scored under a dark-field microscope. The percentage of fluorescing cells was quantitated. In negative controls, specific T-cell and B-cell sera were substituted with DPBS or preimmune rabbit serum. Positive controls included cell suspensions of bursal or thymic cells reacted with homologous sera. Negative control cells uniformly lacked detectable staining, whereas in positive controls, >80% of cells in homologous cell and antiserum combinations expressed intense surface fluorescence. Because of some cross-contamination of bursa with T cells and of thymus with B cells, a minor proportion (usually <5%) of cells in the heterologous cell and antiserum combinations also fluoresced.

RESULTS

Suppression of mitogenic and tumor cell proliferative responses by adherent SC. Results of several experiments reported in Table 1 indicated that the plastic-adherent cells isolated from SC suspensions of normal specific pathogen-free 15 × 7 chickens were inhibitory to PHA-induced blastogenesis of autochthonous SC. The inhibition of the mitogenic response was quite striking, although the extent of inhibition varied between experiments. The inhibition noted was mediated by adherent cells and was not merely due to crowding of the responder cell cultures, because addition of responder cells or of thymocytes in numbers proportional to the adherent cell in responder cell cultures either did not affect or enhanced the mitogenic response of responder cells. In occasional experiments, increasing the number of responder SC in a culture beyond the standard concentration of 10^6 cells resulted in reduced proliferative response to PHA. Results of such experiments were not included in the data. Proliferation of both SC and depleted SC was inhibited by presence of adherent SC. In experiment 4 (Table 1), background counts of certain cell cultures without PHA were higher than expected. High background counts of responder cells in the presence of added autochthonous adherent cells noted in experiment 4 and occasionally in other experiments (e.g., experiments 2 and 3, Table 6) may be due to enhanced viability of responder cells in cultures containing excess macrophages. In certain instances, macrophages condition the cell culture medium by releasing soluble products (4, 25). However, this argument does not apply to elevated background counts in cultures containing depleted SC alone (experiment 4, Table 1), and an explanation for this elevated background activity is not clear.

TABLE 1. *Suppression of mitogenic response by adherent SC*[a]

Expt	Responder cells (10^6)	Inhibiting cells (responder:inhibitor ratio 1:1 or 1:2)	cpm[b]	
			PHA	No PHA
1	Spleen	None	2,790 ± 939	85 ± 3
		Spleen	7,017 ± 382	89 ± 7
		Adherent spleen	347 ± 67	148 ± 29
2	Spleen	None	6,855 ± 772	88 ± 12
		Spleen	14,835 ± 277	167 ± 5
		Adherent spleen	375 ± 160	115 ± 13
3	Depleted spleen[c]	None	6,022 ± 290	197 ± 50
		Depleted spleen	5,591 ± 56	188 ± 3
		Adherent spleen	1,875 ± 299	136 ± 4
		Thymocytes	5,559 ± 218	128 ± 10
4	Depleted spleen[d]	None	4,673 ± 49	124 ± 17
		Depleted spleen	8,975 ± 262	1,501 ± 84
		Adherent spleen	1,657 ± 188	4,293 ± 384
		Thymocytes	8,082 ± 769	212 ± 14

[a] Responder SC were obtained from 4- to 10-week-old 15 × 7 chickens. Inhibiting cells were autologous untreated SC or plastic adherent cells isolated from autologous SC. In experiment 3, autologous thymocytes were also used as inhibitors.

[b] Average counts per minute of [^{125}I]iododeoxyuridine in triplicate cultures ± standard error of the mean.

[c] SC were treated with carbonyl iron.

[d] SC were depleted of macrophages by first treating cells with carbonyl iron and then passing nonphagocytic cells through a rayon column.

Data in Table 2 show that adherent SC were also inhibitory to proliferation of MDCC-MSB-1 cells over a 48-h incubation period. Inhibition of tumor cell proliferation was also specific to the presence of adherent SC and did not result from crowding of tumor cell cultures because unfractionated SC, nonadherent SC, or thymocytes were not inhibitory. Occasionally, as in experiment 4 of Table 2, unfractionated SC were also inhibitory to MDCC-MSB-1 cells, although SC-mediated inhibition was usually not as pronounced as that induced by adherent cells isolated from the same spleen. The inhibitory component in unfractionated spleen was apparently

TABLE 2. *Suppression of MDCC-MSB-1 cell proliferation by adherent SC*

Expt	Inhibiting cells added to 10^4 MDCC-MSB-1 cells (tumor cell:inhibitor cell ratio 1:40 or 1:100)[a]	cpm[b]	% Inhibition
1	None	8,960 ± 261	
	Spleen	9,048 ± 350	0
	Adherent spleen	293 ± 19	97
	Thymocytes	9,316 ± 129	−4
2	None	6,632 ± 280	98
	Adherent spleen	103 ± 5	
	Non-adherent spleen	7,431 ± 146	−12
	Thymocytes	7,504 ± 328	−13
3	None	6,836 ± 263	
	Spleen	7,251 ± 60	−6
	Thymocytes	6,948 ± 3	0
	Adherent spleen	128 ± 10	98
	Spleen + adherent spleen[c]	545 ± 22	92
	Spleen + thymocyte[c]	7,300 ± 65	−7
4	None	10,413 ± 569	
	Spleen	3,903 ± 740	62
	Spleen treated with carbonyl iron and rayon column	11,825 ± 1,420	−13

[a] MDCC-MSB-1 cells were incubated alone or with inhibiting cells obtained from 4- to 10-week-old 15 × 7 chickens. Cultures were pulse-labeled with [^{125}I]iododeoxyuridine for the last 4 h of the 48-h incubation period.

[b] Average counts per minute of [^{125}I]iododeoxyuridine in triplicate cultures ± standard error of the mean.

[c] SC (2×10^5) were mixed with 2×10^5 adherent SC or thymocytes.

the macrophage population, because the inhibitory activity of SC could be removed by removal of adherent and phagocytic cells by pretreatment with carbonyl iron and a rayon column (experiment 4, Table 2).

Effect of macrophage depletion on mitogenic response of normal SC. The intensity of the mitogenic response of SC from normal donor chickens varies extensively. Because results already discussed showed that macrophages resident in the spleen were inhibitory to this response, it was of interest to determine whether suboptimal mitogenic response in some chickens may be due to presence of macrophages and whether removal of inhibitory cells would result in an overall higher mitogen-induced proliferation of normal SC. SC obtained from numerous chickens were stimulated with PHA before and after macrophages were removed. Data on selected experiments in Table 3 show that the response of SC to PHA was not substantially

TABLE 3. *Effect of macrophage depletion on mitogenic response of SC*

Expt	SC treatment	cpm[a]	
		PHA	No PHA
1	None	3,369 ± 319	119 ± 8
	Carbonyl iron	4,440 ± 179	138 ± 6
	Carbonyl iron-rayon column	4,637 ± 395	137 ± 8
2	None	3,691 ± 244	122 ± 3
	Carbonyl iron	4,061 ± 180	141 ± 9
	Carbonyl iron-rayon column	3,961 ± 165	115 ± 6
3	None	9,018 ± 1,169	159 ± 13
	Carbonyl iron-rayon column	7,629 ± 148	145 ± 22
4	None	6,039 ± 263	137 ± 21
	Carbonyl iron-rayon column	8,335 ± 179	159 ± 36

[a] Average counts per minute of [^{125}I]iododeoxyuridine in triplicate cultures ± standard error of the mean.

altered by removal of macrophage from the responding cell population. Thus, the number of macrophages present in normal spleens was suboptimal for affecting suppression. This conclusion was substantiated in the following experiments in which minimum numbers of macrophages needed for optimum suppression were quantitated.

Optimum inhibitor-to-responder cell ratios for maximum suppression. Graded doses of autochthonous adherent cells were added to 10^6 responder SC. Control cultures contained 10^6 responder SC and either additional responder cells or autochthonous thymocytes in numbers corresponding to the number of added adherent cells. The results of two experiments are shown in Fig. 1. Suppression of mitogenic response was not apparent until the ratio of responder to inhibitor cells reached at least 1:1. A ratio of responder cells to inhibitor cells of 1:2 was more inhibitory than one of 1:1. Thus, considerably greater numbers of macrophages were needed to affect suppression than are ordinarily present in normal spleens.

Figure 2 shows data on four experiments in which the number of macrophages needed for optimum suppression of tumor cell proliferation was determined. The results with various adherent cell populations varied. Appreciable suppression was not noted until the MDCC-MSB-1 cells to adherent cells ratio was 1:20; fewer macrophages were not inhibitory. With some adherent cell preparations, tumor cell-to-macrophage ratios of 1:100 or 1:200 were needed for detecta-

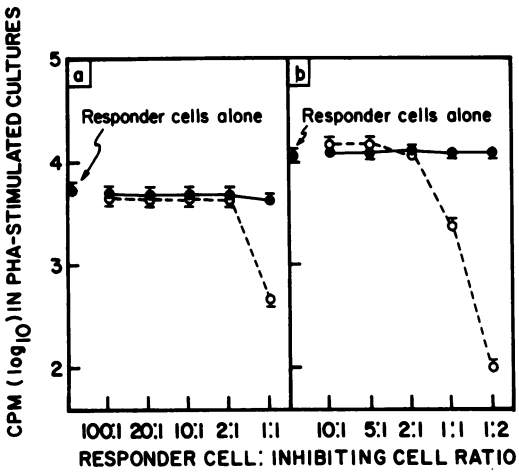

FIG. 1. *Effect of graded numbers of inhibiting cells on mitogenic response of normal SC. The following inhibiting cells were used: (a) ●, SC; ○, adherent SC; (b) ●, thymocytes; ○, adherent SC. Vertical bars represent standard error of the mean.*

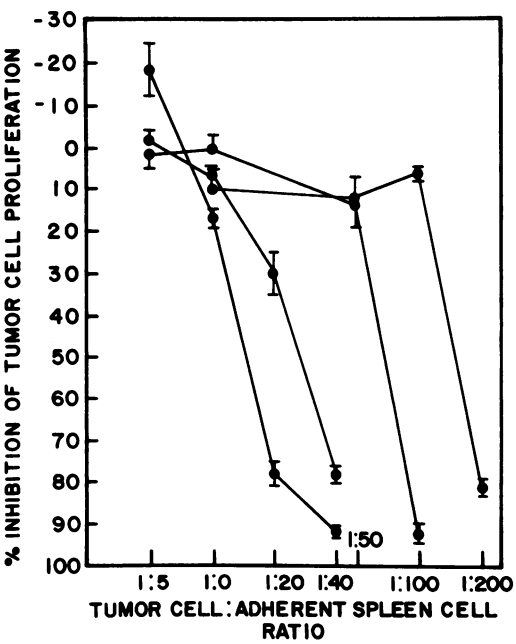

FIG. 2. *Effect of tumor cells:inhibiting cell ratio on inhibition of tumor cell proliferation. Each line represents a separate experiment. Vertical bars represent standard error of the mean.*

ble suppression. The addition of unseparated spleen cells or thymocytes to tumor cell cultures at ratios proportional to those shown in Fig. 2 for adherent cells did not influence tumor cell proliferation.

Suppression of mitogenic response of SC by allogeneic adherent cells. Suppression of MSB-1 cell proliferation by adherent SC from 15 × 7 chickens showed that homology on the major histocompatibility B locus between responders and suppressor cells was not necessary. Although detailed genetic constitution of MDCC-MSB-1 cells has not been published, this cell line was developed in Japan, and donor chickens were expected to be genetically unrelated to the highly inbred 15 × 7 chickens being maintained at our laboratory. It was of interest to determine whether the suppression of the mitogenic response was also independent of allogeneic restriction between responders and inhibitor cells. Results given in Table 4 indicated that responder spleen cells of lines 15 × 7, N, and P with conflicting histocompatibility makeup could be suppressed by syngeneic as well as allogeneic suppressor macrophages.

Identity of suppressor cells. Initially, spleens from several normal 15 × 7 chickens were examined for the proportion of T, B, phagocytic, and adherent cells. Results showed that the proportion of adherent cells in spleens of various chickens examined ranged from 2.0 to 5.2% (Table 5). The proportion of latex-ingesting cells in unseparated spleens varied between spleens and ranged from 2.1 to 8.3%. However, results of a latex ingestion test on adherent cells showed that all adherent SC were not phagocytic. Thus, either some nonphagocytic cells also adhered, or some potentially phagocytic cells lost phagocytic capability after one cycle of adherence. Clearly, a certain proportion of cells that reacted with antisera to T and B cells was also adherent to plastic substrate. In unseparated spleen, the proportion of T, B, and latex-ingesting cells did not add up to 100; thus, all cells present in SC could not be recognized by the test criteria used.

The suppressor cell in adherent cell population was identified as being a macrophage because, as shown in Tables 6 and 7, the suppressor cell activity was not influenced by pretreating adherent cells with highly specific anti-T cell and anti-B cell sera and complement; whereas treatment of adherent cells with the anti-macrophage reagent carrageenan ameliorated the suppressor activity both for mitogen-induced blastogenesis of normal SC (Table 6) and for tumor cell proliferation (Table 7).

DISCUSSION

The present study revealed that spleens of normal specific pathogen-free chickens contained cells that were inhibitory to the in vitro mitogenic response of autochthonous, syngeneic, and allogeneic spleen cells. The target cell of observed suppression was presumably the T cell,

TABLE 4. *Suppression of mitogenic response of SC by allogeneic adherent cells*

Expt	Responder		Inhibitor[a]		cpm[b]	
	Cell	Genetic line	Cell	Genetic line	PHA	No PHA
1	Depleted spleen	15 × 7	None		6,005 ± 33	71 ± 1
			Adherent spleen	15 × 7	62 ± 2	144 ± 8
			Adherent spleen	N	270 ± 94	147 ± 4
			Thymocytes	15 × 7	7,969 ± 330	109 ± 8
	Depleted spleen	N	None		1,138 ± 89	134 ± 35
			Adherent spleen	N	179 ± 18	196 ± 42
			Adherent spleen	15 × 7	110 ± 45	243 ± 54
			Thymocytes	N	2,472 ± 438	270 ± 122
2	Depleted spleen	P	None		7,839 ± 106	173 ± 26
			Adherent spleen	P	1,464 ± 38	733 ± 65
			Adherent spleen	15 × 7	839 ± 39	1,976 ± 785
			Thymocytes	P	14,279 ± 264	195 ± 14

[a] Responder:inhibitor ratio was 1:1 in experiment 1 and 1:2 in experiment 2.
[b] Average counts per minute of [^{125}I]iododeoxyuridine in triplicate cultures ± standard error of the mean.

TABLE 5. *Identification of cells comprising untreated and adherent SC population*

SC preparation no.	Untreated SC (%)				Adherent SC (%)		
	Staining with anti-T cell serum[a]	Staining with anti-B cell serum[a]	Adherent	Latex ingesting	Staining with anti-T cell serum[a]	Staining with anti-B cell serum[a]	Latex ingesting
1	44.0	20.0	3.6	3.7	1.5	34.0	13.4
2	50.0	16.4	2.8	3.3	9.5	18.5	20.6
3	60.5	15.7	5.2	3.9	15.7	19.3	8.0
4	54.4	23.0	3.8	8.3	12.7	21.0	12.5
5	46.5	14.8	Not done	3.7	8.4	26.8	Not done
6	47.5	16.4	2.8	3.0	6.4	27.6	16.9
7	51.0	21.2	4.0	4.0	10.7	19.5	16.1
8	61.4	25.9	2.2	3.9	25.0	8.6	19.0
9	55.4	21.5	3.0	7.1	14.4	8.4	11.2
10	53.8	26.5	2.0	2.1	16.5	20.7	18.8
Mean	52.4	22.4	3.3	4.3	12.1	20.4	15.2

[a] Surface immunofluorescent staining.

because PHA in the chicken induces blastogenesis primarily of the T-cell population (11).

Optimum suppression of PHA response was noted when suppressor cells equaled or exceeded the number of responding cells in the culture. Clear evidence showed that suppression did not result from crowding of mitogen-responsive cells in the culture vessel because the addition of a proportionate number of thymocytes to the culture did not inhibit response. Increasing the number of responding cells in the culture to correspond with the total number of cells in responder and suppressor cell mixtures also, in most experiments, did not decrease PHA response below the level of response of the optimum number of responder cells alone. In occasional experiments, however, raising the number of responder cells above the standard concentration of 10^6 cells per culture resulted in a reduced PHA response. Such experiments were excluded from this study, and the mechanism of reduced mitogenic response of cultures containing high concentrations of responder cells was not investigated. A similar inhibitory effect of high cell concentrations of responder rat SC was reportedly mediated by adherent T cells (9).

Efforts were made to determine the effector cell of observed suppression, and evidence showed that it was most likely a macrophage. Suppressor cells were firmly adherent to plastic substrate and could not be released by repeated vigorous washings with warm medium. The suppressor function was not performed by T cells (9) because suppressor adherent cells isolated from normal spleens had a minor proportion of cells with surface receptors detectable by antiserum that reacted specifically against chicken T cells. Furthermore, the suppressor function of

TABLE 6. *Effect of antisera to T and B cells and of carrageenan on suppressor cells of mitogenic response*

Expt	Responder cells (10^6)	Inhibiting cells (10^6)	Inhibiting cell treatment	cpm[a]	
				PHA	No PHA
1	Depleted spleen	None		6,102 ± 284	115 ± 5
		Depleted spleen	None	11,863 ± 517	369 ± 41
		Thymocytes	None	9,844 ± 815	248 ± 33
		Adherent spleen	None	653 ± 63	235 ± 6
		Adherent spleen	Anti-T cell serum + complement	2,872 ± 83	211 ± 4
2	Spleen	None		17,453 ± 752	249 ± 8
		Adherent spleen	None	1,001 ± 102	2,301 ± 114
		Adherent spleen	Anti-B cell serum + complement	260 ± 21	793 ± 199
3	Spleen	None		7,604 ± 1,922	2,055 ± 166
		Thymocytes	None	14,747 ± 377	282 ± 51
		Adherent spleen	None	1,561 ± 232	4,040 ± 277
		Adherent spleen	Carrageenan	8,906 ± 704	3,461 ± 141

[a] Average counts per minute of [^{125}I]iododeoxyuridine in triplicate cultures ± standard error of the mean.

TABLE 7. *Effect of anti-T cell serum and carrageenan on suppression of tumor cells proliferation by adherent SC*

Expt	Inhibiting cells added to 10^4 MDCC-MSB-1 cells (tumor cell:inhibitor cells ratio 1:100)	Inhibiting cell treatment	cpm[a]	% Inhibition
1	None		17,103 ± 442	
	Depleted spleen	None	15,020 ± 981	12
	Adherent spleen	None	3,345 ± 224	80
	Adherent spleen	Anti-T cell serum + complement	3,979 ± 321	77
2	None		10,492 ± 764	
	Thymocytes	None	12,865 ± 45	−23
	Adherent spleen	None	3,860 ± 981	63
	Adherent spleen	Carrageenan	12,372 ± 320	−18

[a] Average counts per minute of [^{125}I]iododeoxyuridine in triplicate cultures ± standard error of the mean.

adherent cells could be reduced or eliminated by treatment with the antimacrophage agent carrageenan but not with antiserum to T and B cells in the presence of complement. The adherent suppressor cells were not uniformly phagocytic when incubated in vitro for 2 to 3 h in medium containing an abundance of latex particles. Poor phagocytic activity of adherent cells either could be due to suboptimum conditions used for testing phagocytosis or more likely could be due to the fact that adherent cells were obtained from normal chickens that were not deliberately stimulated with antigens and the adherent cell represented a population of nonactivated macrophages, with diverse morphological and functional characteristics (43).

Macrophages have been implicated in suppression of normal cellular as well as humoral immune functions (32). Macrophage-mediated suppression of mitogenic response is particularly well pronounced in mammalian hosts undergoing neoplastic disease (5, 10, 15, 34, 46). Tumor-inducing viruses in chickens have also been implicated in reduced in vitro mitogenic response of lymphoid cells (3, 6, 7, 20, 21, 24, 27, 38, 44), and we have recently reported that mitogenic hyporesponsiveness in Marek's disease-affected chickens is mediated by suppressor macrophages (21). In light of the presence of suppressor macrophages in uninfected control chickens, we need to determine whether suppressor macrophages detectable after infection with Marek's disease virus are qualitatively or quantitatively different from those present in normal chickens.

Adherent SC from normal chickens, in addition to suppressing mitogenic response, also were cytostatic for rapidly proliferating cells of a line derived from an Marek's disease lymphoma (MDCC-MSB-1 line). The term "cytostasis" is used provisionally here; we did not determine whether macrophages were also cytolytic for MDCC-MSB-1 cells. The inhibitor cells of tumor cell proliferation appeared similar in character to the suppressor cells of the mitogenic response, i.e., the inhibitor cells were adherent and could be inactivated by carrageenan but not by treatment with antisera to T cells. Macrophage-mediated cytostasis of tumor cells in vitro has also been noted in mammalian models, al-

though this activity is best performed by macrophages that have undergone activation (13, 26). Lee et al. (21) reported that spleen cells from chickens infected with Marek's disease virus inhibited in vitro proliferation of MDCC-MSB-1 cells. We do not know whether the mechanism of tumor cell inhibition by macrophages from Marek's disease-affected chickens was similar to the inhibition mediated by normal macrophages. Furthermore, the in vivo role of cytostatic macrophages in tumor surveillance is not entirely clear at the moment. Because inhibitory cells were present in normal chickens and because this cytostatic effect is generally nonspecific (14), the inhibitory cells may play a role in natural defense against tumor development, and this natural role may be enhanced by preexposure of the host to tumor cells or antigens. The nonspecific cytostatic activity of macrophages seems to become more prominent in tumor-bearing animals (31).

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