MECHANISMS OF GENETIC RESISTANCE TO FRIEND VIRUS LEUKEMIA

III. Susceptibility of Mitogen-Responsive Lymphocytes Mediated by T Cells*

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Several oncogenic viruses are profoundly immunosuppressive in vivo (1). The mechanism by which the suppression of immunocompetent cell function is brought about is not entirely clear. In view of the important role of suppressor cells in regulating normal immune response, attention has been focused recently on the possible role of suppressor cells in mediating oncornavirus-induced immunosuppression. In the Moloney sarcoma virus model, both B cells (2) and macrophages (3) have been ascribed a suppressor role. In the Friend virus (FV)[1] erythroleukemia model, it has been suggested that the leukemic cells themselves may act to suppress immune responses in vitro (4, 5).

In the accompanying paper we presented evidence that FV, when added in vitro, can suppress the mitogenic response of T and B cells from mouse strains susceptible to FV leukemogenesis (6). Such mice are also extremely susceptible to the suppression of T- and B-cell functions when infected by FV in vivo. Lymphoid cells from mice genetically resistant to FV leukemia and immunosuppression in vivo are resistant to FV-induced suppression of mitogenesis in vitro. This resistance has been shown to be abrogated when mice were treated with ^{89}Sr, which selectively destroys marrow-dependent (M) cells without affecting T-cell, B-cell, or macrophage functions (7, 8).

In this paper we have analyzed the mechanism by which FV suppresses T- and B-cell mitogenesis in vitro. We conclude that FV mediates its suppressive effect on T- and B-cell mitogenesis through a suppressor cell. The suppressor cell requires thymic influence for maturation, bears Thy-1 antigen, adheres to nylon wool, and is sensitive to lysis by cortisol. Such cells are present in the lymphoid tissues of mice susceptible to leukemogenesis in vivo and to in vitro suppression of mitogenesis induced by FV. In mice resistant to Friend leukemia, the suppressor cell function as tested in vitro seems to be lacking. Since treatment of resistant (*Fv-2^{rr}*) mice with ^{89}Sr renders their lymphocyte mitogenesis suscepti-

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[1] *Abbreviations used in this paper:* anti-Ig, rabbit antimouse immunoglobulin (7S); Con A, concanavalin A; DS, dextran sulfate; FFU, spleen focus-forming units; FV, Friend virus; LPS, lipopolysaccharide (*Salmonella typhi*); M, marrow-dependent.

ble to FV-induced suppression (6), it appears that M cells suppressible by ^{89}Sr regulate the function of suppressor cells in mice genetically resistant to Friend erythroleukemia.

Materials and Methods

Mice. Mice of the strains BALB/c congenic at the *nu* locus (*nu*/+, *nu*/*nu*, and +/+) and C3H/He were bred in our own animal facility. C57BL/6 (B6) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. All mice were 5 to 6 wk old when used for experiments.

Cell Separation Techniques. For purification of T cells the nylon wool filtration technique described originally by Julius et al. (9) and modified by Trizio and Cudkowicz (10) was used. Cells adherent to glass wool were separated by preparing a glass wool column (10), and rinsed with 50 ml of RPMI 1640 medium. 150 × 10^6 spleen cells in 2 ml were loaded onto a previously rinsed column and allowed to incubate at 37°C for 30 min. The nonadherent cells were then gently washed off with 20 ml of cold medium. The filtered cells were washed twice in cold medium and used. Such a suspension was practically devoid of any macrophages (less than 1%) as assessed by ability to ingest latex beads. Separation of macrophages from spleen cell suspensions was also done by incubation with carbonyl iron followed by exposure to a magnetic field (3). In each experiment, a pool of three spleens were used as a common source of treated and untreated cells.

Treatment of Cells with Rabbit Antimouse Immunoglobulin (Anti-Ig) and Complement (C). This was done as described by Gorzyncki (2). Anti-Ig was purchased from Cordis Laboratories, Miami, Fla. Preliminary experiments indicated that treatment of spleen cells with a 1:15 final dilution of the anti-Ig and guinea pig C completely abolished the response to the B-cell mitogen lipopolysaccharide (LPS) without affecting the response to concanavalin A (Con A). This dilution was then used in experiments.

Treatment of Cells with Anti-Thy 1 Serum. AKR anti-C3H Thy-1 serum (lot no. 18) was purchased from Amersham/Searle Corp., Chicago, Ill. Preliminary experiments to characterize its potency indicated that treatment of spleen cells with a 1:60 final dilution abrogated the mitogenic response to Con A without affecting the response to LPS. Spleen or thymus cells were treated with a 1:60, 1:40, or 1:5 final dilution of heat-inactivated antiserum at 4°C for 1.5 h followed by washing and further incubation at 37°C with a 1:10 final dilution of guinea pig C.

Administration of Cortisol. Mice were injected intraperitoneally with 2.5 mg of hydrocortisone acetate 2 days before sacrifice and preparation of thymus cell suspensions. Control mice were injected with the vehicle, saline. The methods of ^{89}Sr administration, lymphocyte cultures, and calculation of data were described in the previous paper (6).

Results

Effect of FV on Mitogenesis of Nylon-Filtered Spleen Cells from Susceptible Strains of Mice. The mitogenic response of splenic T and B cells of mice susceptible to FV in vivo is suppressed by addition of FV into the culture (6). Since the suppressed response of spleen and lymph node cells to mitogens and viral antigens from mice bearing Moloney sarcomas have been ascribed to suppressor macrophages and B cells (2, 3), we decided to investigate if the FV-induced in vitro suppression of Con A response was also mediated by similar suppressor cells. As a first step, we separated adherent cells from C3H or BALB/c spleen cells by three different techniques: iron magnet treatments, filtration over rayon wool columns, and by passage over glass wool. It is generally accepted that these techniques deplete the spleen of macrophages, and in the case of rayon wool, some B cells (3). These procedures did not in anyway affect suppressibility of the C3H and BALB/c spleen cells, suggesting that macrophages were not essential for mediating the suppressive effect of FV on lymphocyte mitogenesis (Table I; exps. 1–3).

TABLE I

Effect of Various Treatments on the Suppressibility of T-Cell Mitogenesis by FV

Exp.*	Cells	Δ Blastogenesis		Suppression
		No FV	+FV (200 FFU/well)	
		mean cpm ± SE		%
1	Whole spleen	36,038 ± 167	16,561 ± 436	55
	Iron magnet	30,907 ± 583	15,945 ± 513	49
	Rayon filtered	51,177 ± 524	27,744 ± 669	46
2	Whole spleen	63,580 ± 1,205	33,581 ± 926	48
	Nylon wool filtered	112,710 ± 1,650	101,666 ± 1,551	10
	Glass wool filtered	97,408 ± 1,374	46,814 ± 171	52
3	Whole spleen	31,808 ± 261	13,759 ± 339	57
	Nylon wool filtered	61,288 ± 702	55,588 ± 440	10
	Glass wool filtered	43,321 ± 232	23,031 ± 380	47
4	Whole spleen	43,562 ± 901	15,849 ± 655	64
	Nylon wool filtered‡	87,702 ± 894	77,775 ± 907	12
5	Whole spleen	45,197 ± 1,260	25,927 ± 808	43
	Anti-Ig + C§	50,798 ± 829	22,023 ± 772	57

Results: Nylon wool-filtered spleen cells were significantly ($P < 0.05$ determined by Student's t test) less suppressed than whole spleen cells in exps. 2-4. Suppression of iron magnet-treated (exp. 1), rayon-filtered (exp. 1), glass wool-filtered (exps. 2 and 3), and anti-Ig-treated (exp. 5) spleen cells was not significantly different ($P > 0.05$) from that of whole spleen cells.

* BALB/c spleen cells used in exps. 3 and 4, C3H spleen cells in other experiments.

‡ Nylon wool-filtered spleen cells, were free of any phagocytic cells (measured by phagocytosis of latex beads) and contained less than 3% EAC rosetting cells.

§ Rabbit antimouse IgG (1:15), guinea pig serum (1:10), and 10^7 spleen cells/ml exactly as described (2).

We next looked for suppressor cells by a different approach. Since T cells which respond to Con A do not adhere to nylon wool, we tested whether or not mitogenesis of nylon-purified T cells would be suppressed by FV. If the Con A-responsive T cells were directly suppressed by FV, then the effects of FV on unfiltered (whole) spleen cells and nylon-filtered spleen cells should be identical. However, if the effect of FV on T cells was mediated through suppressor cells, and not directly on Con A-responsive T cells, purified populations of T cells could become resistant to suppression by FV. As indicated by the representative experiments in Table I, suppression of T-cell mitogenesis by FV was almost completely abrogated when nylon-filtered (purified) T cells were used. Since filtration over glass wool is the first step in the purification of T cells by nylon wool (10), the suppressor cell, missing in the final purified T-cell preparation, could have adhered either to glass wool or nylon wool. To distinguish between these two possibilities, we took glass wool-filtered spleen cells and tested a sample for suppression by FV. The remaining cells were filtered further over nylon wool. The suppressibility of whole spleen cells, glass wool-filtered, and nylon wool-filtered spleen cells was then compared. Glass wool filtration did not affect the suppressibility of C3H and BALB/c spleen cells by FV (Table I; exps. 2

and 3). Therefore, the suppressor cells must have adhered to nylon wool and not to glass wool.

Effect of Lysing B Cells by Anti-Ig and C on the Suppressibility of Con A-Responsive Spleen Cells by FV. In the process of purification of T cells by nylon wool filtration, the lymphoid cells which adhere to nylon wool are macrophages, B cells, and a certain number of T cells. Our reasons for concluding that some T cells also adhere to nylon wool are discussed later. Any cells which adhere to nylon wool would have to be considered as candidates for suppressor cell activity. Since macrophages seemed not to be the suppressor cells in this system (as discussed above), the next step was to determine if B cells were suppressors. If this were true, then elimination of B cells from spleen cell suspension would be expected to render the Con A response of T cells refractory to suppression by FV. Therefore, BALB/c spleen cells were treated with anti-Ig and C. This treatment completely abolished the mitogenic response to the B-cell mitogen LPS without affecting the response to Con A. The Con A responses of these cells were suppressed by FV as much as the untreated spleen cells (Table I; exp. 5). This indicated that the B cells are not necessary for FV-induced suppression of T-cell mitogenesis in vitro.

Effect of Adding Whole Spleen Cells on the Suppressibility of Nylon-Filtered Cells. If nylon-filtered spleen cells were missing a suppressor cell population which was present in the unfractionated spleen cells, it would be logical to expect that addition of whole spleen cells to nylon-filtered cells would restore suppressibility to the latter population. This prediction was confirmed in the following experiments. Nylon-filtered BALB/c spleen cells were mixed with varying ratios of whole spleen cells (1:1, 5:1, 10:1, and 15:1 filtered:whole spleen cells). The effect of adding 200 spleen focus-forming units (FFU) of FV on the Con A response of the mixtures was investigated. As low a frequency as 10% of whole spleen cells in the presence of 90% nylon-filtered cells made the latter suppressible by FV (Table II; exps. 1 and 2). Controls consisted of 100% unfractionated spleen cells which were fully susceptible to suppression, and 100% nylon-filtered cells which were refractory to suppression. These experiments also excluded the possibility that increased concentration of T cells after nylon wool filtration was responsible for observed resistance to FV.

The Nature of the Suppressor Cells in Whole Spleen Cells. The fact that a small number of whole spleen cells could restore suppressibility to nylon-filtered cells indicated that whole spleen cells provided the suppressor cell missing in the nylon-filtered cells. We therefore decided to use this mixture protocol to investigate the nature of the suppressor cell present in the whole spleen. From previous experiments, macrophages and B cells seemed unlikely to be suppressor cells. Therefore T cells had to be considered as potential candidates. We believed that some T cells were being retained on the nylon column for the following reasons. The frequency of T cells (Thy-1 positive) is approximately 30% in the spleen (11). Cell yields after nylon wool filtration were in the range of 15–20% and the filtered cells were contaminated by 2–3% erythrocyte antibody complement rosette-forming cells (B cells). Thus, approximately $18\% \times 100 \div 30 = 60\%$ of the splenic T cells were being recovered by nylon filtration. A considerable proportion of T cells was adhering to nylon wool.

TABLE II

Effect of Adding Whole Spleen Cells on the Supressibility of Nylon-Filtered Spleen Cells

Exp.	Cells	Δ Blastogenesis		Suppression
		No FV	+FV (200 FFU/well)	
		mean cpm ± SE		%
1	Whole spleen (W)	35,933 ± 935	13,795 ± 395	62
	Nylon wool filtered (NF)	66,118 ± 815	60,254 ± 689	9
	NF + W (1:1)	51,144 ± 693	22,928 ± 992	56
2	W	47,834 ± 721	21,783 ± 906	55
	NF	96,871 ± 1,042	90,618 ± 897	7
	NF + W (5:1)	78,413 ± 658	37,762 ± 307	52
	NF + W (10:1)	94,817 ± 471	43,846 ± 178	54
	NF + W (15:1)	97,231 ± 1,554	61,653 ± 369	37
3	W (nu/+)	32,363 ± 1,011	17,056 ± 346	48
	NF (nu/+)	49,912 ± 873	46,292 ± 636	8
	NF (nu/+) + W (nu/+) (10:1)	53,545 ± 601	24,028 ± 569	56
	NF (nu/+) + W (nu/nu) (10:1)	50,647 ± 574	46,976 ± 181	8

Result: NF spleen cells were less suppressible ($P < 0.05$) than whole spleen cells (exps. 1–3). NF + W mixture of spleen cells were significantly ($P < 0.05$) more suppressed than NF spleen cells (exps. 1–3), except in exp. 3 when nu/nu W spleen cells were used.

To investigate if T cells were suppressor cells in our system, we determined whether or not whole spleen cells from athymic nude mice could provide suppressor cells capable of restoring suppressibility to nylon-filtered spleen cells. Whole spleen cells from *nu/+* or *nu/nu* mice (on BALB/c background) were mixed with nylon-filtered BALB/c spleen cells in a ratio of 1:10. The cells were cultured with Con A and with or without FV as before. While *nu/+* whole spleen cells restored suppressibility to nylon-filtered cells, *nu/nu* spleen cells failed to do so (Table II; exp. 3). Thus, nude mice apparently lack suppressor cells.

In analogous experiments, we asked the question if the suppressor cells could be lysed by anti Thy-1 serum and C. Whole spleen cells were treated with a 1:60 final dilution of anti-Thy-1 serum and C. They were then mixed with nylon-filtered spleen cells to see if they would restore suppressibility. Anti-Thy-1-treated whole spleen cells could still provide suppressor function in the mixture (Table III; exp. 1). This experiment was performed twice and each time a 1:60 concentration of anti-Thy-1 serum completely abolished the Con A response of the treated spleen cells, but did not remove the suppressor cells. This result indicated either that the suppressor cells were not T cells or that the cells did not express enough Thy-1 antigens to be lysed by the concentration of anti-Thy-1 serum used. The former postulate seemed unlikely in view of the results with nude mice; therefore, the latter possibility was tested in the next experiment.

Spleen cells were treated with a 1:40 or a 1:5 dilution of anti-Thy-1 serum (plus C) before being washed and later added to nylon wool-filtered cells as sources of suppressor cells. Cells treated with the more dilute (1:40) anti-Thy-1 serum and C provided suppressor function, but cells treated with the more concentrated

TABLE III

Effect of Anti-Thy-1 Treatment on the Ability of Spleen and Thymus Cells to Restore Suppression of Nylon-Filtered Spleen Cells

Exp.	Cells	Anti-Thy-1 serum final concentration	Δ Blastogenesis		Suppression
			No FV	+FV (200 FFU/well)	
			mean cpm ± SE		%
1	W (whole spleen)	–	27,295 ± 475	13,235 ± 387	52
	NF (nylon filtered)	–	49,024 ± 1,515	43,469 ± 680	12
	NF + W*	–	45,945 ± 397	22,596 ± 921	51
	NF + W	1:60	46,716 ± 154	20,627 ± 970	56
	W‡	1:60	1,195 ± 89	–	–
2	W	–	6,440 ± 139	3,678 ± 426	43
	NF	–	12,590 ± 876	12,920 ± 1,012	0
	NF + W*	–	10,018 ± 422	5,635 ± 351	44
	NF + W	1:40	10,956 ± 1,219	4,783 ± 654	57
	NF + W	1:5	12,420 ± 656	12,701 ± 117	0
	W‡	1:40	645 ± 69	–	–
	NF + thymus*	–	10,198 ± 327	4,636 ± 116	55
	NF + thymus	1:40	13,330 ± 770	12,810 ± 1,916	4
	NF + thymus	1:5	10,453 ± 1,820	9,010 ± 1,799	14
	Thymus‡	1:40	275 ± 92	–	–

Result: NF spleen cells were significantly less ($P < 0.05$) suppressed than W spleen cells, whereas the mixture of untreated W spleen cells and NF spleen cells were as suppressible ($P > 0.05$) as W spleen cells (exps. 1 and 2). The NF spleen cells mixed with thymus cells treated with 1:5 or 1:40 dilution of anti-Thy-1 serum or mixed with W spleen cells treated with 1:5 (but not 1:40 or 1:60) dilution of anti-Thy-1 serum were less suppressible ($P < 0.05$) than W spleen cells.

* In all experiments the ratio of nylon-filtered cells to whole spleen or thymus cells was 10:1.

‡ Controls for the efficacy of anti-θ treatment.

(1:5) antiserum and C lost suppressor function (Table III; exp. 2). This result indicated that the suppressor cells in the spleen were very weakly Thy-1 positive. In the same experiment, we also used whole thymus cells as a source of suppressor cells. Thymus cells (10%), like spleen cells, also provided the suppressor cells for nylon-filtered spleen cells (Table III; exp. 2). Treatment of thymus cells with either 1:40 or 1:5 dilution of anti-Thy-1 serum and C eliminated suppressor cell function.

Our next effort in the characterization of suppressor T cells was to determine if they were sensitive to cortisol. Most T cells in the mouse thymus are extremely sensitive to lysis by cortisol, but the small percentage of cortisol-resistant T cells seems to be capable of performing most of the T-cell functions (12). Thymus cells were harvested from control BALB/c mice as well as mice treated with 2.5 mg cortisol 2 days earlier. These cells were then cultured with Con A and the effect of added FV was tested. Unlike the previous experiments, the presence of suppressor cells was looked for by the effect of FV on the mitogenesis of cortisol resistant thymocytes directly and not by using the mixture protocol. In each of three experiments the mitogenesis of thymocytes from cortisol-treated animals was quite resistant to suppression by FV, whereas the Con A response of thymocytes from control BALB/c mice was profoundly

suppressed (Fig. 1). This phenomenon was highly reproducible, indicating that the suppressor cell in the thymus is very susceptible to lysis by cortisol. In similar experiments when spleen cells of cortisol-treated BALB/c mice were exposed to Con A and FV, the proliferation was suppressed (Fig. 1), indicating that the splenic suppressor cell was not lysed by cortisol.

Resistance of Nude Mice to Suppression of B-Cell Mitogenesis by FV. FV can suppress both B- and T-cell mitogenesis in vitro (6). Experiments thus far have indicated that FV-induced suppression of T-cell mitogenesis is mediated by a T-suppressor cell lacking in nude mice. If the B-cell mitogenesis is suppressed by a similar T-suppressor cell, then B cells from nude mice would be expected to be relatively resistant to FV-induced suppression of mitogenesis. The effect of FV on the B-cell proliferation induced by dextran sulfate (DS) and LPS on spleen cells from *nu/nu*, *nu/+*, and +/+ mice was compared. Mitogenesis of B cells from nude mice was quite resistant to suppression while cells from *nu/+* and +/+ mice were quite susceptible (Table IV).

Effect of Nylon Wool Filtration on the Susceptibility of Spleen Cells from B6 Mice Treated with ^{89}Sr. Previous experiments indicated that T-suppressor cells mediated the FV-induced suppression of lymphocyte mitogenesis when

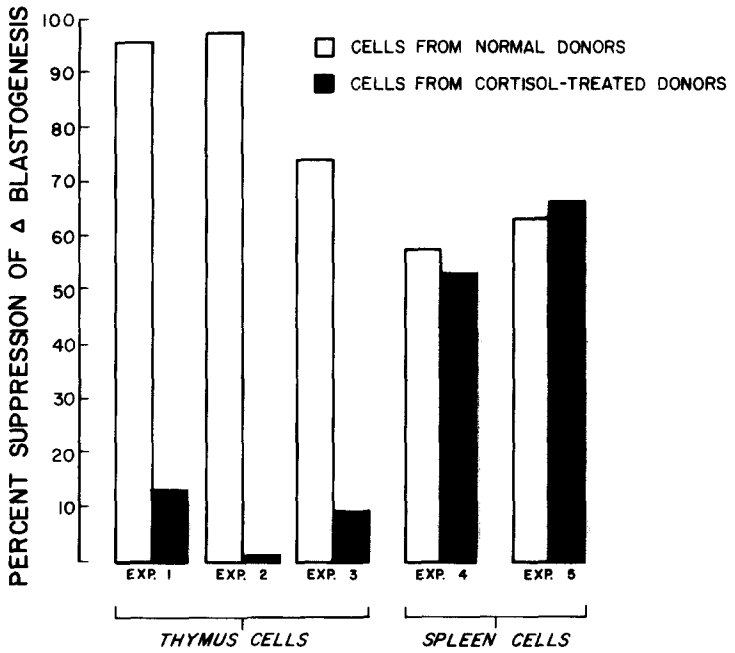

FIG. 1. Effect of cortisol on the suppressibility of thymic and splenic T cells by FV. BALB/c mice were injected with 2.5 mg hydrocortisone acetate 2 days before sacrifice. Spleen and thymus cells from control and cortisol-treated donors were cultured with Con A and the effect of FV on T-cell mitogenesis was tested directly by adding 200 FFU of FV into the cultures. The degree of suppression of the Con A response was the index of suppressor cell activity in lymphoid cells from normal vs. cortisol-treated donors. Result: thymus cells from cortisol-treated donors were markedly resistant to FV-induced suppression while spleen cells were unaffected.

TABLE IV
Effect of FV on the B-Cell Mitogenesis of Spleen Cells from Nude Mice

Exp.	Donor genotype	Mitogen	Δ Blastogenesis		Suppression
			No FV	+FV (200 FFU/well)	
			mean cpm ± SE		%
1	+/+	LPS	8,906 ± 141	3,846 ± 105	57
	nu/+	LPS	10,452 ± 407	5,092 ± 108	52
	nu/nu	LPS	10,996 ± 788	10,164 ± 253	8
2	+/+	DS	7,914 ± 299	3,019 ± 167	62
	nu/+	DS	8,766 ± 390	4,664 ± 421	47
	nu/nu	DS	12,156 ± 1,019	11,004 ± 746	10

Result: Spleen cells from *nu/nu* mice were significantly less suppressible ($P < 0.05$) than spleen cells from *nu/+* or +/+ mice.

spleen cells were taken from BALB/c mice, which are susceptible to FV in vivo. We next proceeded to ascertain if the mechanism of suppression of lymphocyte mitogenesis by FV was similar in spleen cell suspensions of B6 mice previously treated with ^{89}Sr. We first tested whether spleen cells from B6 mice treated with ^{89}Sr contained suppressor cells adherent to nylon wool or contained mitogen-responsive cells directly suppressible by FV. Spleen cells from ^{89}Sr-treated B6 mice were filtered over nylon wool and then the filtered and unfiltered cells were stimulated with Con A in the presence or absence of FV. Spleen cells from control B6 mice were also tested. The nylon-filtered spleen cells of the ^{89}Sr-treated B6 mice were refractory to FV-induced suppression of T-cell mitogenesis (Table V). This result supports the suppressor cell concept and excludes the possibility that in ^{89}Sr-treated mice the Con A-responsive lymphocytes become directly suppressible by FV.

Effect of FV on Mixtures of Spleen Cells from Control and ^{89}Sr-Treated B6 Mice. To confirm the hypothesis that ^{89}Sr treatment increases the numbers and/or function of suppressor cells within the spleen of B6 mice, we determined whether mixtures of spleen cells from control and ^{89}Sr-treated B6 mice were susceptible to FV-induced suppression. The ratios used were 3:1 and 9:1 control:^{89}Sr treated. This experiment is analogous to mixing nylon-filtered and whole spleen cells of BALB/c mice in similar ratios. The mixtures of spleen cells were quite susceptible to suppression of mitogenesis by FV (Table V). This result indicates that elimination of M-cell function in B6 mice results in the appearance of suppressor cells not normally present in their spleens. This experiment also indicates that M cells do not function in vitro against T-suppressor cells during the culture period. Otherwise, the M cells of the control B6 mice may have prevented the function of suppressor cells from spleens of ^{89}Sr-treated mice.

Discussion

The mitogenic response of normal T cells and B cells from various lymphoid organs of mice susceptible to FV leukemia is suppressed by addition of FV in vitro (6). Suppression of the mitogenic response is under genetic control and

TABLE V
Detection of suppressor cells in spleen of ^{89}Sr-treated B6 mice

Exp.	Mouse pretreatment	Δ Blastogenesis		Suppression
		No FV	+200 FFU FV/well	
		mean cpm ± SE		%
1	None (control)	32,878 ± 1,001	30,666 ± 5,095	7*
	^{89}Sr	29,273 ± 1,498	17,167 ± 89	42
	^{89}Sr (NF)‡	59,837 ± 4,315	62,245 ± 466	−4*
2	None (control)	17,906 ± 1,440	17,186 ± 1,644	5*
	^{89}Sr	56,453 ± 6,859	22,910 ± 2,763	60
	3:1 Control: ^{89}Sr§ spleen cells	45,273 ± 3,620	20,633 ± 2,340	55
	9:1 Control: ^{89} Sr§ spleen cells	30,716 ± 969	16,086 ± 1,038	48

Results: Whole spleen cells from B6 mice treated with ^{89}Sr were significantly more suppressible than whole spleen cells from control B6 mice or NF spleen cells from ^{89}Sr-treated B6 mice (exp. 1). Mixtures of spleen cells from control and ^{89}Sr-treated B6 mice were significantly ($P < 0.05$) more suppressible than spleen cells from control B6 mice (exp. 2).

* Not significantly different from control (No FV).

‡ NF, nylon filtered.

§ Spleen cells from ^{89}Sr-treated and control B6 mice were mixed in the ratios indicated before culture.

lymphoid cells from mice resistant to FV leukemia are refractory to suppressive effects of FV in vitro. However, such resistance is lost when M cells are eliminated by treatment of mice with ^{89}Sr (6).

A scheme to describe our findings and to present our concept of the mechanism of suppression of lymphocytes by FV in vitro is presented in Fig. 2. The working hypothesis we wish to develop and defend in the discussion that follows has three components: (*a*) mitogen-responsive B and T cells are not directly suppressed by FV; (*b*) a T cell is the direct target of FV and functions as a suppressor of mitogen-responsive lymphocytes; and (*c*) the numbers and/or function of these T-suppressor cells in hemopoietic tissues of the mouse are influenced by M cells in vivo but not during the in vitro culture period. It follows that the genes which determine the in vitro resistance or susceptibility of lymphocytes to FV control properties of M cells and not T-suppressor cells or mitogen-responsive lymphocytes.

Evidence presented in this paper supports the view that mitogen-responsive T and B cells are not intrinsically susceptible to the suppressive effects of FV in vitro. Thus, splenic T cells from genetically susceptible mice were rendered resistant to FV-induced suppression of mitogenesis after filtration over nylon wool. This finding alone could also mean that there are two subsets of mitogen-responsive T cells, one susceptible to suppression by FV and retained by nylon

M cells —Regulate numbers and/or function of→ T-suppressor cells —Acted upon by FV to suppress proliferation of→ Mitogen-responsive T and B lymphocytes

FIG. 2. A proposed mechanism of immunosuppression by FV.

wool, and the other resistant to FV and not retained by nylon wool. However, the restoration of suppressibility to nylon wool-filtered cells by addition of 10% normal (whole) spleen cells rules out the above possibility. In the 1:10 mixture of whole spleen cells and nylon-filtered cells, at least 90% of the Δ blastogenesis values were contributed by the nylon-filtered T cells; the 50% reduction in Δ blastogenesis values after treatment of the mixture with FV (Table II) indicates that addition of normal spleen cells affected the suppressibility of the nylon-filtered T cells. The possibility that nylon filtration in someway altered the surface of T cells so as to make them resistant to FV is also rendered unlikely by the mixture experiment. The mixture experiment also is inconsistent with the possibility that nylon purification merely concentrated T cells, so that the same dose of virus which could suppress whole spleen cells was not enough to suppress the nylon-filtered pure T-cell population. Thus a suppressor cell, which adheres to nylon wool, mediates the suppression of lymphocytes by FV.

Physical separation of suppressor T cells from other T cells in other model systems has met with limited success. Suppressor cells in rat spleens have been reported to be weakly adherent to glass wool (13). In our model, the suppressor cells were definitely not adherent to glass wool. Cells capable of suppressing antibody synthesis have also been separated by histamine-coated beads (14). We have not determined if the suppressor cells in the FV system have histamine receptors.

Several characteristics of the suppressor cell which mediates FV-induced in vitro suppression have been defined. The suppressor cells require thymic influence for maturation since spleen cells from nude mice failed to provide suppressor cells for nylon-purified *nu*/+ spleen cells (Table II; exp. 3). The suppressor cell bears Thy-1 antigen since anti-Thy-1 and C treatment of thymocytes and spleen cells eliminated the suppressor function when these cells were mixed with nylon wool-filtered cells. However, the ease of lysis by anti Thy-1 serum differed in the case of spleen and thymus. While treatment with a 1:40 dilution of anti Thy-1 serum lysed the suppressor cell in the thymus, it failed to do so in the spleen. A much higher concentration of anti-Thy-1 serum, i.e. 1:5 dilution, was required to remove the suppressor cell from spleen cell suspension. The high dilution (1:60) which failed to lyse the suppressor cells of the spleen completely abolished the Con A response. These results indicate that T-suppressor cells are present both in the thymus and the spleen; after migration from the thymus to the spleen the cells apparently express less surface Thy-1 antigen. Loss of surface Thy-1 antigen is known to occur when T cells in the thymus mature and seed the peripheral lymphoid organs (15). It is difficult to be certain if the T-suppressor cells themselves respond to Con A stimulation. This possibility, however, seems unlikely since treatment of spleen cells with 1:60 dilution of anti-Thy-1 serum abolished the Con A response but not the suppressor cell function.

Since the B-cell mitogenic responses of cells from the spleen and the marrow were also suppressed by FV, it is tempting to speculate that the same or similar population of T-suppressor cells responsible for suppression of Con A-responsive cells also mediate suppression of B cells. The resistance of B cells of nude mouse spleen to suppression by FV (Table IV; exps. 1 and 2) strongly supports this

suggestion. Recent observations indicate that T cells are present in the mouse bone marrow (16). The bone marrow T-cell population may contain the suppressor T cells which mediate the FV-induced suppression of DS-responsive B cells. The bone marrow T cells are only weakly Thy-1 positive (16), which is consistent with our suggestion that T-suppressor cells lose most of their surface Thy-1 antigen after migration from the thymus.

Another interesting correlation is seen between the amount of surface Thy-1 antigen, degree of differentiation, and susceptibility to lysis by cortisol. Treatment of BALB/c mice with cortisol made their thymocyte mitogenesis resistant to suppression by FV, but did not affect to any significant degree the suppressibility of splenic T cells in vitro. We interpret this to mean that T-suppressor cells in the thymus are sensitive while those in the peripheral lymphoid organs are resistant to cortisol. It has previously been shown that sensitivity to lysis by cortisol is related to the amount of surface Thy-1 antigen (17). Thus, the differential sensitivity to cortisol correlates very well with the differential sensitivity of the splenic and thymic suppressor cells to lysis by anti-Thy-1 treatment. Another difference between the splenic and thymic T-suppressor cells is the lack of adhesion of thymic T-suppressor cells to nylon wool. In experiments not reported here, we failed to separate T-suppressor cells from the Con A-responsive cells of thymocyte cell suspensions by nylon wool filtration.

The nature of interaction between FV and the T-suppressor cell is not yet clear. Heat-inactivated or UV-irradiated FV preparations lose immunosuppressive activity in vitro, indicating that live and replicating virus is required. Since UV inactivation preserves the ability of virus to adsorb and penetrate into cells, it follows that mere adsorption and penetration into the cell are not enough to "activate" the suppressor cells. Once the suppressor cell is activated, it may secrete soluble mediators. Alternatively, cell contact between activated suppressor cells and mitogen-responsive cells may be required. Preliminary experiments have failed to detect any soluble mediator in virus-infected spleen cell cultures. The role of cell contact in mediating suppression is under investigation. It is also conceivable that the suppressor cells function by supporting virus replication.

By defining the suppressor cell, we gained some insight into the mechanism of genetic resistance of B6 mice to the in vitro immunosuppressive effects of FV. B6 mice lose their genetic resistance to suppression by FV in vitro after treatment with the bone-seeking isotope, ^{89}Sr. In view of the fact that T and B cells per se are not the direct target of the suppressive effects of FV, the acquired susceptibility of cells of B6 mice after ^{89}Sr treatment must be explained in terms of suppressor cell functions. Two possibilities were considered: (*a*) normal B6 mice do not possess enough suppressor cells to mediate the effect of FV because M cells suppressible by ^{89}Sr restrain their proliferation; and (*b*) normal B6 mice do possess the suppressor cells which are eliminated by M cells after FV infection in vitro. The latter possibility would imply that M cells would have to be present in lymphoid cell suspension in a functional state. A preliminary distinction between these two possibilities was permitted by the experiment in which spleen cells from normal B6 mice were mixed with cells from ^{89}Sr-treated B6 mice (Table V; exp. 2). Since 10% spleen cells from ^{89}Sr-treated B6 mice con-

ferred susceptibility on 90% normal B6 spleen cells, it is very likely that spleen cells from ^{89}Sr-treated B6 mice provided the suppressor cells normally missing from the spleens of adult B6 mice. The resistance of normal B6 spleen cells could not have been due to M-cell-mediated rejection of activated T-suppressor cells in vitro since in the mixtures enough M cells would have been provided by the normal B6 spleens to reject the small numbers of additional suppressor cells provided by the ^{89}Sr-treated donor. We conclude, therefore, that M cells serve to regulate the number of T-suppressor cells in vivo. In mice with lymphocyte populations genetically resistant to FV in vitro, the number of suppressor cells is maintained at a low level, thus affording them protection from immunosuppression, in vitro and perhaps also in vivo.

The M cells which are important in Friend leukemogenesis are not necessarily identical to the cells which mediate marrow allograft rejections. The common feature between the two systems is the susceptibility to ^{89}Sr (7, 8). Recently, we have also found that M cells are important in early host defense against *Listeria monocytogenes* (18). It is conceivable that all these functions are mediated by M cells of different specificities.

Summary

Friend leukemia virus (FV) suppressed the proliferative responses of spleen, lymph node, marrow, and thymus cell populations to various T- and B-cell mitogens. Cells taken from mice, e.g. BALB/c genetically susceptible to leukemogenesis in vivo were much more susceptible to suppression of mitogenesis in vitro than similar cells from genetically resistant mice, e.g., C57BL/6. Nylon wool-purified splenic T cells from BALB/c and C3H mice lost susceptibility to FV-induced suppression of mitogenesis but became suppressible by addition of 10% unfiltered spleen cell. Thus, FV mediates in vitro suppression of lymphocyte proliferation indirectly by "activating" a suppressor cell.

The suppressor cell adhered to nylon wool but not to glass wool or rayon wool columns. Pretreatment of spleen cells with carbonyl iron and a magnet did not abrogate the suppressor cell function. Suppressor cells were not eliminated by treatment with rabbit antimouse immunoglobulin (7S) and complement (C). However, high concentrations of anti-Thy-1 plus C destroyed suppressor cells of the spleen; thymic suppressor cells were much more susceptible to anti-Thy-1 serum. Nude athymic mice were devoid of suppressor cells and their B-cell proliferation was relatively resistant to FV-induced suppression in vitro. The suppressor cells in the thymus (but not in the spleen) were eliminated by treatment of mice with cortisol. Thus, FV appears to mediate its suppressive effect on mitogen-responsive lymphocytes by affecting "T-suppressor cells."

Spleen cells from C57BL/6 mice treated with ^{89}Sr to destroy marrow-dependent (M) cells were much more suppressible by FV in vitro than normal C57BL/6 spleen cells. However, nylon-filtered spleen cells of ^{89}Sr-treated C57BL/6 mice were resistant to FV-induced suppression in vitro, indicating that the susceptibility of spleen cells from ^{89}Sr-treated B6 mice is also mediated by suppressor cells. Normal B6 splenic T cells were rendered susceptible to FV-induced suppression of mitogenesis by addition of 10% spleen cells from ^{89}Sr-treated B6 mice. Thus, M cells appear to regulate the numbers and/or functions of T-suppressor cells which in turn mediate the immunosuppressive effects of FV in

vitro. Neither mitogen-responsive lymphocytes nor T-suppressor cells are genetically resistant or susceptible to FV. The genetic resistance to FV is apparently a function of M cells, both in vitro as well as in vivo.

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