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Enhanced Antibody Response in Retrovirus-Infected Mice Treated with Endotoxin or Nontoxic Polysaccharide Derivative (42613)

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Abstract. Friend leukemia virus (FLV) is a retrovirus which causes marked suppression of the immune response of genetically susceptible mice. In the present study the depressed antibody response to sheep erythrocytes by spleen cells from FLV-infected mice was partially reversed by injection of either a bacterial endotoxin or a nontoxic polysaccaride derivative directly into infected mice or by addition to spleen cell cultures from these mice immunized *in vitro* with sheep red blood cells (SRBC). The endotoxin and PS in a dose-related manner markedly increased the antibody responsiveness of the spleen cells to SRBC. Thus these results indicate that the nontoxic polysaccharide derivative has properties equivalent to the toxic endotoxin in enhancing the antibody responsiveness of FLV-suppressed spleen cells to a T-cell-dependent antigen like SRBC.

Infection of genetically susceptible mice with a murine retrovirus such as Friend leukemia virus (FLV) results in marked impairment of immunocompetence such as antibody formation or cell-mediated immunity. For example, studies in this and other laboratories have shown that FLV, when given to susceptible mice, markedly depresses the function and activity of B and T lymphocytes to a wide variety of antigens (1–3). Suppressed activity also occurs with macrophages from FLV-infected mice, including depressed antigen degradation and presentation as well as mediator production (4, 5). Moreover, a number of studies have shown that endotoxins, i.e., the toxic lipopolysaccharide (LPS) component of the cell walls of gram-negative bacteria, may be highly immunostimulatory, especially when given together with an antigen (6–8). Recent studies in this laboratory have also shown that endotoxin has immunorestorative effects in FLV-infected mice (3, 9). The results of the present study show that not only intact endotoxin, which contains the lipid A moiety considered the major contributor to the toxicity of the molecule, but also the polysaccharide (PS)-rich derivative of endotoxin which does not contain lipid A has the ability to enhance antibody formation in FLV-infected mice, both *in vivo* and *in vitro.* Such enhancement depended on the dose of the bacterial product and the time after infection with virus.

Materials and Methods. *Experimental animals.* Inbred male Balb/c mice, 6–8 weeks of age at the start of an experiment, were obtained from Cumberland View Farms (Clinton, TN). The mice weighed between 18 and 20 g at the start of an experiment and were housed in groups of 6 to 10 in plastic mouse cages under pathogen-free conditions. The animals were fed Purina mouse pellets and water *ad libitum.*

Leukemia virus and infection. The mice were infected by intraperitoneal (ip) injection of 100 infectious doses (ID_{50}) of FLV contained in 0.1 ml of a 1% clarified homogenate of infected spleen cells (10). The virus was maintained as described previously by passage through adult Balb/c mice and contained both the spleen focus forming and the lymphatic leukemia virus components of the complex. The stock virus preparation contained no detectable lymphocytic choriomenigitis or lactic dehydrogenase virus (10).

Antigen and immunization. Sheep red blood cells (SRBC) in Alsever's solution were obtained from BBL (Cockeysville, MD). The red cells were washed several times in sterile saline and resuspended to a concentration of 0.5% erythrocytes. For *in vivo* immunization

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mice were injected ip with 0.5 ml saline containing 4×10^8 SRBC.

In vitro culture and immunization. Covered plastic Linbro plates (24 wells) were used as culture chambers as described previously (11). Spleen cells from normal or virus-infected mice were washed in RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) containing 10% fetal calf serum and 100 μg each penicillin and streptomycin per milliliter. The numbers of viable nucleated cells were determined by the trypan blue dye exclusion technique with a hemocytometer. A suspension of 5×10^6 viable spleen cells in 2.0 ml medium was added to each well in the Linbro plates and cultured for 5 days at 37°C in a humidified chamber containing 5% CO_2 and 95% air. For immunization *in vitro* 0.1 ml of a 0.1% suspension of SRBC was added to each culture (i.e., 2×10^6 erythrocytes).

Assay for antibody-forming cells. The number of direct hemolytic antibody plaque-forming cells (PFC) or SRBC was determined by the standard micromethod as described previously (12). The numbers of PFCs were enumerated for at least three to five cultures prepared from the spleen of at least three to four mice per group and the average number of PFCs per 10^6 cells was calculated. In all cases only direct nonfacilitated plaques were enumerated and these were considered due to 19S immunoglobulin M antibody-producing cells.

Endotoxin and PS preparations. Serratia marcescens endotoxin was prepared by the trichloroacetic acid extraction procedure as described previously (13–15). The nontoxic smaller molecular weight PS-rich derivative containing less than 0.1% lipid and less than 1% amino acids was prepared by acid hydrolysis as described previously (14). The PS was not toxic for mice even at a dose of 2.0 mg per animal and showed no endotoxic activity when tested by the chick embryo lethality or by Limulus lysate coagulation activity, both considered highly sensitive for Lipid A toxicity of endotoxin.

Results. Studies in this laboratory have shown that spleen cells from FLV-infected mice become markedly deficient in their ability to produce antibody to SRBC. It is apparent in Table I that spleen cells from normal mice after immunization with SRBC showed a significant antibody response but those from mice injected with FLV and immunized with sheep erythrocytes showed a progressive decline in their ability to produce antibody to the SRBC as a function of time after infection. Within 1–2 weeks after infection there was a marked depression of the ability of the spleen cells from infected mice to produce antibody. By Days 25–30 after infection there was essentially little or no detectable antibody response. However, when the mice were injected with 10–50 μg endotoxin on the day of infection there was a significant antibody response. Although injection of 10 μg endotoxin resulted in only a slight to moderate affect on the immunosuppression induced by FLV, the 50-μg dose had a marked enhancing effect, especially when

TABLE I. EFFECT OF ENDOTOXIN OR NONTOXIC POLYSACCHARIDE DERIVATIVE ON ANTIBODY FORMATION BY FLV-INFECTED MICE

In vivo treatment[a] (μg/mouse)	PFC/10^6 spleen cells[b]			
		FLV infected mice challenged on Days[c]		
	Normal mice	7–10	15–20	25–30
None	530 ± 10	160 ± 48	32 ± 12	10
Endotoxin, 10	680 ± 160	210 ± 40	48 ± 20	26 ± 10
50	1350 ± 215	686 ± 132	320 ± 32	62 ± 30
PS, 10	545 ± 65	262 ± 38	73 ± 16	40 ± 12
50	1040 ± 120	620 ± 16	312 ± 13	58 ± 22

[a] Groups of mice injected ip with 4×10^8 SRBC and indicaed dose of endotoxin or PS.
[b] Average PFC response ± SD for six to eight mice per group 5 days after immunization with SRBC.
[c] Mice injected ip with 100 ID_{50} FLV on day indicated before challenge immunization.

TABLE II. EFFECT OF ENDOTOXIN OR NONTOXIC POLYSACCHARIDE DERIVATIVE *in Vitro* ON ANTIBODY FORMATION BY SPLEEN CELLS FROM FLV-INFECTED MICE

In vitro addition to cultures (μg/culture)[a]	Antibody PFC response[b]					
	Normal mice			FLV-inected mice[c]		
	Per 10^6 spleen cells	Percentage of control	*P*	Per 10^6 spleen cells	Percentage of control	*P*
None (control)	685 ± 43	—	—	146 ± 41	—	—
Endotoxin, 5.0	1236 ± 126	180	<0.05	256 ± 56	175	<0.01
10.0	1754 ± 138	256	<0.01	322 ± 98	221	<0.01
20.0	1590 ± 145	232	<0.01	565 ± 152	387	<0.01
PS, 5.0	1150 ± 68	168	<0.05	396 ± 130	271	<0.01
10.0	1532 ± 141	224	<0.01	626 ± 162	429	<0.01
20.0	1456 ± 136	213	<0.01	512 ± 94	351	<0.01

[a] Cultures of splen cells from normal or infected mice treated with indicated concentrations of endotoxin or PS and immunized with 2×10^6 SRBC.
[b] Antibody PFC response per 10^6 spleen cells ± SD for 8–24 cultures per group 5 days after culture.
[c] Donor mice injected 10–12 days earlier with 100 ID_{50} FLV.

the animals were tested within the first week or so after infection.

The nontoxic PS at equivalent doses had essentially similar effects as the larger molecular weight and more toxic endotoxin. Injection of 50 μg PS, similar to LPS, resulted in a much greater antibody response in the 7- to 10-day-infected mice as compared to the 10-μg dose and also resulted in higher antibody responses in mice infected for longer periods of time, although the response continued to decrease (Table I). Thus PS, as well as endotoxin, resulted in greater stimulation of antibody formation by spleen cells at the highest dose used, i.e., 50 μg.

The enhancing effect of these bacterial products on depressed antibody responsiveness of spleen cell from FLV-infected mice was even more evident in completely *in vitro* assays. For these experiments mice were infected with FLV, the spleen obtained at autopsy and cultured *in vitro* with an immunizing dose of sheep SRBC. As is apparent in Table II, there was a marked depression of antibody formation by spleen cells from infected mice immunized *in vitro* with SRBC. However, treatment of the cells with either endotoxin or PS resulted in a dose-dependent increase in the PFC response *in vitro*. The 20-μg dose of either endotoxin or PS was optimal in enhancing the PFC responses of spleen cells from 10- to 12-day FLV-infected mice. The relative increase in antibody responsiveness was higher with spleen cells from FLV-infected mice as compared to control mice. This appeared due to the initial lower number of antibody-producing cells capable of responding to SRBC in the spleen cell cultures from infected mice as compared to cultures prepared from normal mice. However, the total number of PFCs in treated cultures from infected mice did not approach the optimal response evident with spleen cells from normal mice treated with the same dose of bacterial product. Nevertheless, the increase in the antibody response of spleen cells from infected mice was consistently enhanced and, moreover, was essentially similar with both endotoxin and PS. In addition, as is apparent in Fig. 1, the kinetics of the antibody response enhanced by these preparations or PS were generally similar throughout the 6 days of culture. The response seen on Day +4 or +5 after culture immunization was also evident earlier and later during the culture period, i.e., Day +2 as well as Day +6.

Discussion and Conclusions. Endotoxins have been studied for the last few decades in terms of their adjuvant activity *in vivo* and *in vitro* (7, 8, 14, 15). The endotoxin from many different gram-negative bacteria is known to enhance the immune response to a wide variety of antigens when administered

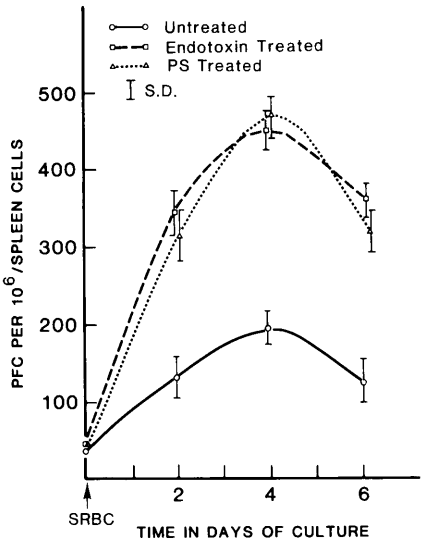

FIG. 1. Cytokinetics of the antibody response by spleen cells from FLV-infected mice immunized *in vitro* with sheep erythrocytes after treatment with endotoxin or PS. Each point represents the average antibody response of spleen cell cultures from three to four mice infected with FLV 10–12 days earlier and cultured *in vitro* at a concentration of 5×10^6 cell/ml with 2×10^6 SRBC as antigen. The solid line represents FLV-infected cells and the dashed and dotted lines indicate cell cultures treated with 10 μg endotoxin or PS at the time of culture initiation.

together with an antigen. However, it may also modulate the immune response as evident by suppressed responses when given prior to or at a later period of time afte antigen. Most studies concerning the immunomodulatry effects of endotoxins have utilized crude bacterial preparations rich in the toxic lipid A moiety (8, 14). Such preparations were found in previous studies, as well as in the present one, to be an adjuvant for spleen cells from leukemic animals (5, 9, 16). However, the toxicity of the product may have some disadvantage since individuals who are immunocompromised may be more susceptible to microbial toxins as compared to normal individuals.

The polysaccaride-rich derivative is free of detectable toxicity even when administered at doses 100 times greater than those used in the present studies. Thus it was of interest to determine whether the nontoxic polysaccharide which also is known to have adjuvant properties could enhance the immune responses of spleen cells from FLV-infected mice which show marked impairment of immune responsiveness to a wide variety of antigens, including erythrocytes. It is clear from the results of the present study that either endotoxin or PS, in equivalent doses, enhanced the antibody response to sheep RBCs both *in vivo* and *in vitro* by spleen cells from FLV-infected as well as noninfected mice. The greatest enhancement occurred early after infection, when there was only a partial or moderate suppression of the response of spleen cells to SRBC. Later in the course of infection, i.e., 2–3 weeks or more, there was a much greater suppression of the immune response and the adjuvants were less effective in enhancing antibody formation. Nevertheless, even spleen cells from these animals showed increased antibody formation to SRBC.

SRBC is considered a T-cell-dependent antigen but antibody formation to such antigen depends not only on fully functioning B cells but also on helper and regulatory T cells as well as macrophages. It is now recognized that FLV infection may inhibit not only B cells and their precursors, but also T lymphocytes, including both effector cells involved in cell mediated immunity and regulatory cells involved in antibody formation (1, 17). FLV also markedly affects macrophages, decreasing their functional activity as phagocytic cells as well as their ability to serve as accessory cells for antibody formation and to produce various mediators of immunity such as interleukins and interferons (4, 5).

It is not yet known how endotoxin stimulates antibody formation even in normal lymphoid cell populations, let alone by FLV-infected cells. However, it is believed that it may serve as a macrophage activator, resulting in release of various mediators such as interleukins and interferons which have a positive effect on the antibody response mechanism of the host. Endotoxin may also directly influence not only B lymphocytes, but also T cells. All three cell types appear to be defective in FLV-infected animals, including B cells or their progeny involved directly in antibody formation. Thus it is possible that endotoxin may influence the de-

pressed response of spleen cells from virus-infected mice by directly interacting with remaining immunocytes or their precursors, increasing their functional activity as well as stimulating those cells which have not yet been infected by the virus.

Many studies to date have suggested that much of the endotoxin's activity is due to the lipid A moiety (8), although studies in this and other laboratories have suggested that the polysaccharide-rich preparation free of lipid has beneficial effects (14). As shown in the present study, the nontoxic polysaccaride also enhanced the antibody response of spleen cells from FLV-infected mice. Since the polysaccharide is free of the toxic activity of the parent material, it is possible that this bacterial product may be useful in restoring immunocompetence in retrovirus-infected individuals with acquired immunodeficiency (18). Although the mechanisms involved are not yet known, it seems likely that further studies with nontoxic polysaccharide derivatives should provide important information as how such smaller molecular weight products from bacterial endotoxins influence the immune response not only by normal lymphoid cells but also by lymphoid cells suppressed by retrovirus infection.

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