The Pharmacologic Effects of PGE₁ on Murine Lymphocytes

By Alan Winkelstein and Vicki E. Kelley

Although PGE₁, in pharmacologic quantities, possesses significant in vivo immune-modulating activities, its effects on different lymphoid populations have not been defined. In the present study, lymphocyte numbers, turnover rates, and functional activities were assessed in Swiss-Webster mice receiving PGE₁, 200 μg twice daily, for up to 14 days. The most pronounced cellular alterations were observed in the thymus. After 2 wk, the number of theta-positive cells decreased by 81%. Total splenic cellularity was concomitantly reduced by 28%. This was primarily due to a loss of B lymphocytes; the number of these cells decreased by 40%. Bone marrow lymphocytes were also decreased by PGE₁ administration. By contrast, peripheral blood lymphocytes and splenic T cells were not significantly altered. Similar changes in lymphocyte numbers were observed in adrenalectomized mice, indicating that depletion was not due to a corticosteroid effect. In normal mice, PGE₁ reduced the in vivo incorporation of ³HThd in both the thymus and the spleen by approximately 50%; this finding, in conjunction with histologic and cytologic observations, suggests a decrease in intrinsic rates of lymphopoiesis. Residual splenocytes were not impaired in their in vitro responses to three polyclonal mitogens: PHA, Con-A, and endotoxin. Studies in cortisol-treated mice showed that PGE₁ caused a decrease in both steroid-sensitive and steroid-resistant thymocytes and splenocytes.

PGE₁, in pharmacologic quantities, has proven capable of altering many in vivo immune responses. However, changes in immunologic reactivity vary greatly with the experimental model. Some test responses are impaired, whereas others may be augmented. Of considerable interest, recent studies indicate that PGE₁ in animal models can ameliorate three immunologically related diseases. It can suppress manifestations of adjuvant arthritis in rats, reduce glomerular damage in murine model of acute serum sickness nephritis, and prolong survival in NZB/W mice. The effect results from a retardation in the development of the immune complex glomerulonephritis.

The cellular basis for these immune-modulating activities has not been defined. In particular, there are no comprehensive studies detailing the effects of in vivo administration on numbers or functional activities of lymphocytes. Therefore, the present investigations were undertaken to systematically evaluate PGE₁-induced lymphocytic changes in both normal and corticosteroid-treated mice. Our results suggest that this compound causes selective cellular loss of thymocytes, bone marrow lymphocytes, and splenic B cells. By contrast, peripheral blood and splenic T cells are largely unaffected.

MATERIALS AND METHODS

Female, Swiss-Webster mice, 6–10 wk of age, were used in all experiments. PGE₁ was kindly provided by Dr. J. E. Pike, Upjohn Company, Kalamazoo, Mich. The compound was dissolved in 10% ethanol-phosphate buffered saline solution and administered subcutaneously in doses of 200 μg twice daily for periods of up to 14 days. Controls received the buffer alone. Both groups of animals were weighed at the beginning and at the end of the experimental protocol. Groups of 8–37 animals were killed, by cervical dislocation, on days 7, 10, and 14 of treatment, and 7 and 14 days after discontinuation of drug administration. Pilot studies employed 14-day courses of PGA and PGF₂α; 9 animals in each group were analyzed after 14 days of administration.

In all animals, the thymus and the spleen were removed in toto and freed of surrounding tissue; single-cell suspensions were prepared by thoroughly mincing the tissues. This was followed by repeatedly aspirating the suspensions through 16- and 22-gauge needles to produce a uniformly dispersed suspension of single cells. Total cellularity in each organ was determined by standard hemocytometer counts.

T lymphocytes in the spleen and thymus were enumerated by modifications of the technique of Raff et al. Cells were incubated with mouse anti-theta serum (Bionetics, Kensington, Md.) that had been absorbed against mouse red cells. Guinea pig complement (Grand Island Biological Company, Grand Island, N.Y.) was added, and the cytotoxicity was determined by the uptake of trypan blue dye. Prior to testing, optimal concentrations of each reagent were determined; for both the anti-theta serum and complement, these proved to be 1:10 dilutions. The total number of T cells was determined by multiplying the theta-positive fraction by the total number of thymic or splenic cells.

B lymphocytes in the spleen were measured by the complement rosette technique. Sheep red cells were sequentially sensitized with a 1:50 dilution of specific rabbit hemolysin (Flow Laboratories Inc., Rockville, Md.) followed by incubation with a 1:100 dilution of mouse complement. Spenic lymphocytes were centrifuged over a Ficoll-Hypaque gradient, washed, and incubated with a 50:1 ratio of sheep red cells to lymphocytes for 30 min at 37°C. Rosettes were enumerated after addition of 0.5% toluidine blue dye. No rosette formation occurred in the absence of either specific antibody or complement.

Bone marrow lymphocytes were measured as follows. Both

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femurs were removed and freed of adherent tissues. The right femur was cut at the epiphyseal plates, and using a 21-gauge needle, the marrow cells were aspirated into 2 ml EDTA-phosphate buffered saline solution. Cell counts were performed by standard hemocytometer methods. The left femur was unroofed, and smears were made using a fine-tipped paint brush, saturated with a solution of EDTA-phosphate buffered saline containing 5% mouse serum. Smears were stained with Wright-Giemsa stain, and a 300-cell differential count performed. Counts were expressed as lymphocytes per femur. Blood lymphocyte counts and differentials were performed using routine hematologic techniques.

In order to assess the potential role of endogenous corticosteroids as a mediator of PGE1 activities, adrenalectomized animals were also evaluated. These studies were performed within 3 days after the surgical procedure. Prostaglandin-treated and control mice were maintained with corticosterone (compound B), 8 μg i.p. twice daily; in the absence of these supplemental steroids, PGE1 treatment was lethal. All animals were sacrificed after 14 days. Using the assay systems described above, thymic T cells and splenic T and B cells were enumerated.

The in vivo thymic and splenic incorporation of tritiated thymidine (3Htdr, New England Nuclear, Boston, Mass., specific activity 6.7 Ci/mM) were measured using modifications of techniques previously reported. The isotope uptake was determined at 1 and 4 hr following an i.p. injection of this specific precursor of DNA. A weighed sample of muscle was similarly processed; this value was used as the background count. Isotope uptake in the thymus and spleen was expressed as counts per minute per milligram (cpm/mg) of tissue; this was corrected by subtracting the values obtained from the muscle sample. Results were also calculated on a basis of uptake per organ and per 106 cells; these values paralleled those calculated per unit weight. In the PGE1 experiments, animals were treated with 200 μg twice daily for 2 days; at the time of the last injections of prostaglandins, the animal received this isotope. Results from 7–10 animals per point were used to calculate an average incorporation.

In vitro responses of splenic cells to polyclonal mitogens were measured in a modified microculture test, adapted from our previously reported assay. Cells were cultured in concentrations of 4 × 105 cells per microriter well in media RPMI 1640 with HEPES buffer (Grand Island Biological). The media was supplemented with 5% fetal calf serum, glutamine (300 μM), gentamicin (60 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin (0.25 μg/ml). The pH was adjusted to 7.2 by addition of 10% NaHCO3 solution. All cultures were performed in triplicate. These were incubated for 3 days at 37°C in a humidified CO2 atmosphere, and 3Htdr, 0.1 μCi/well, was added during the final 18 hr incubation. Phytohemagglutinin (PHA) mitogenic responses were assessed at three dilutions; those for concanavalin A (Con-A) and endotoxin (LPS) at two dilutions. A stock solution of PHA (Wellcome Research Laboratories, Beckenham, England) was diluted 1:5, 1:10, and 1:25 with media; cultures containing 10 μl of each solution were tested in each assay. The final concentrations of Con-A (Sigma Laboratories, St. Louis, Mo.) were 2 μg/ml, 1 μg/ml, and 0.5 μg/ml; those for LPS (S. typhosa 0901, Difco Laboratories, Detroit, Mich.) were 1 μg and 0.5 μg/culture. The Con-A responsiveness of thymus cells was also studied at concentrations of 1 μg, 0.5 μg, and 0.25 μg/culture.

At the termination of the incubation period, cultures were harvested by collecting cells on filter paper using a semiautomatic lymphocyte harvester (Adaps, Denton, Mass.). Isotope uptake was measured by adding Unogel (Schwarz-Mann, Orangeburg, N.Y.) and counting in a Packard tri-carb liquid scintillation counter. Responses were expressed as the net uptake of isotope, which represents the difference between activity in stimulated and unstimulated cultures. In determining the reactivity for each animal, the mitogenic concentration showing the greatest net isotope uptake was used; this concentration varied from animal to animal.

Additional experiments were performed using hydrocortisone in conjunction with PGE1. Test animals received a 14-day course of this prostaglandin. One PGE1-treated group received a single injection of hydrocortisone, 2.5 mg/mouse, 2 days prior to sacrifice; the other received saline. Total cellularity of the thymus and numbers of T and B cells in the spleen were determined using the techniques described above.

In order to assay circulating levels of PGE1, groups of 3 mice per point were injected subcutaneously with 200 μg and sacrificed at 5, 15, 30, 45, and 120 min later. Controls received buffer alone. Endogenous prostaglandin synthetase was blocked with indomethacin, 10 mg/kg, given intraperitoneally 30 min prior to the PGE1 injection. Animals were killed by cervical dislocation, plasma obtained and frozen until analysis. The plasma concentrations of PGE1 were determined as follows. Tritiated PGE1 (approximately 1500 cpm) was added to 150-μl aliquots of plasma to correct for recovery losses. The plasma was diluted with 1 ml of saline and the pH adjusted to 3.5 with formic acid. The sample was extracted twice with ethylacetate (3 ml), and the ethylacetate evaporated under nitrogen. The dried extracts were redissolved and applied to a silicic acid column, and the prostaglandin E fraction was eluted. The prostaglandin-E-like material (i-PGE) was determined by radioimmunoassay after conversion to prostaglandin B using 0.1 N KOH in methanol. Standard curves were constructed using prostaglandin B1.

RESULTS

PGE1, in the concentrations employed in these studies, produced few systemic effects. During the 14-day treatment period, both control and experimental animals experienced a slight weight loss. However, in both groups, the weight loss averaged less than 2 g, and there was no significant difference between treated and control mice. Animals receiving PGE1 generally appeared healthy; the only obvious manifestations were a brief period of lethargy and diarrhea. These abnormalities were transient, lasting less than an hour.

To determine the disappearance of injected PGE1, groups of mice were treated with 200 μg and sacrificed at intervals from 5 min to 2 hr. Endogenous production of prostaglandins was inhibited by indomethacin given 30 min prior to the drug. Analysis of the PGE1 solution indicated that 15% had been converted in vitro to PGA1. Following subcutaneous injection, high blood levels of PGE1 were observed throughout the period of observation (Table 1). The disappearance rates conformed to a biphasic curve; the t1/2 for the first component was 29 min and for the second, 90 min.

Changes in lymphoid populations were sequentially evaluated in both thymic and splenic cell suspensions. Initial studies showed that there were no changes in cellularity after 1 or 2 days of PGE1; animals treated
for these intervals were not further evaluated. However, longer courses of drug administration led to reductions in selective populations of lymphoid cells. The most striking cellular alterations were observed in the thymus. After 14 days, the total thymic cellularity was reduced by 80% (Table 2). There was a corresponding decrease in the number of theta-positive cells (81% decrease). The reduction in thymocytes proved to be a transient phenomenon; within 7 days after discontinuing PGE_1, the number of cells in this organ had returned to normal.

Changes in splenic lymphocytes were less pronounced (Table 3). PGE_1 administration caused a modest reduction in total cellularity; after 14 days, the decrease averaged 28%. However, a differential effect on T and B lymphocytes was observed. Although there was a corresponding decrease in the number of theta-positive cells (81% decrease). The reduction in thymocytes proved to be a transient phenomenon; within 7 days after discontinuing PGE_1, the number of cells in this organ had returned to normal.

Histologic examination confirmed the selective loss of lymphoid cells in mice receiving PGE_1. The overall architecture of the thymus was maintained, but there was a prominent decrease in cortical cellularity. Medullary thymocytes were reduced, but to a lesser extent. There was no evidence of increased cell death, as manifested by excess numbers of degenerated cells, infiltration by neutrophils or monocytes, or nuclear fragments in reticuloendothelial cells. In the spleen, the cellular depletion was primarily restricted to the peripheral regions of the periarteriolar sheaths and to the red pulp; these areas are predominantly populated by B lymphocytes. The inner aspect of the periarteriolar sheaths zones (areas populated by T cells), were preserved. Again, there was no histologic evidence of increased cell death. Additional data, obtained by examining thymic and splenic cell suspensions by trypan blue dye exclusion, indicated that there was no increase in the proportion of nonviable cells. Thus, the histologic and cytologic studies suggest that the effects of PGE_1 were not mediated by a lymphocytotoxic activity.

Lymphoid cells in the peripheral blood and bone marrow were also measured after 14 days of PGE_1 administration. There was no significant change in the blood compartment; the mean lymphocyte count in PGE_1-treated mice was 4708 ± 105 compared to 5267 ± 200 in controls. By contrast, PGE_1 led to a significant reduction in marrow lymphoid cells. The average number of marrow lymphocytes in controls was 6.0 ± 0.6 x 10^6 cells/femur. After 2 wk of drug administration, the number of these cells was reduced to 3.7 ± 0.4 x 10^6, a 38% decrease (p < 0.005).

As PGE_1 is known to augment corticosteroidogenesis,22,23 additional studies were performed to evaluate its activities in adrenalectomized mice. In the absence of supplemental steroids, prostaglandin administration proved lethal to these mice. For maintenance therapy, both controls and PGE_1-treated animals received corticosterone, 8 μg, twice daily. This dose, by itself, produced minimal changes in lymphoid cellularity (Table 4). Of particular note, after 14 days of PGE_1 treatment, there was a significant decrease in splenic cellularity. After 14 days, the number of cells in the spleen was reduced by 40%, which was not significantly different from controls. By contrast, B lymphocytes were reduced by 72%. However, the residual number of cells remained in the spleen. In the thymus, the cellular depletion was primarily restricted to the peripheral regions of the periarteriolar sheaths and to the red pulp; these areas are predominantly populated by B lymphocytes. The inner aspect of the periarteriolar sheaths zones (areas populated by T cells), were preserved. Again, there was no histologic evidence of increased cell death. Additional data, obtained by examining thymic and splenic cell suspensions by trypan blue dye exclusion, indicated that there was no increase in the proportion of nonviable cells. Thus, the histologic and cytologic studies suggest that the effects of PGE_1 were not mediated by a lymphocytotoxic activity.

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administration, the cellular changes in thymocytes and splenocytes were similar to those observed in normal animals. The only difference was that in the adrenalectomized mice, there was a concomitant decrease in splenic T cells. This population was not reduced in normal animals. These data indicate that the reduction in lymphoid populations observed after prostaglandin administration cannot be attributed to a corticosteroid effect.

To further evaluate mechanisms responsible for the prostaglandin-induced decrease in lymphocytes, the in vivo incorporation of 3HTdr into thymic and splenic tissues was measured in both control and treated mice. As shown in Fig. 1, PGE1 administration was associated with reduced isotope incorporation into both tissues. The mean values, expressed as 3HTdr content per milligram, in the treated groups were decreased by approximately 50%; this difference was observed at the two time intervals. Similar results were obtained if incorporation was computed on the basis of total number of thymic and splenic cells or the weights of these organs.

Splenic lymphocytes from animals treated with PGE1 were evaluated for their in vitro response to three polyclonal mitogens: PHA, Con-A, and LPS. As shown in Fig. 2, there were no significant reductions in these parameters. In fact, a modest increase in Con-A activity was observed; the responses to the two other mitogens were equivalent to controls. It should be noted that the isotope uptake in unstimulated cultures from PGE1-treated mice did not differ from that observed in controls. Responses in stimulated cultures were assessed using different concentrations of mitogens; no differences between treated and control mice were observed in the quantity required to elicit maximum reactivity. To further define the effect of PGE1, the Con-A responses of thymocytes were also measured. In parallel with the results of spleen cultures, net isotope incorporation of 3HTdr in thymocytes from PGE1-treated animals did not differ from controls.

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**Table 4. Effects of PGE1 in Adrenalectomized Mice**

<table>
<thead>
<tr>
<th></th>
<th>Adrenalectomized Controls (x 10^6)</th>
<th>PGE1-Treated Mice (x 10^6)</th>
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<tr>
<td>Thymus cells</td>
<td>174 ± 19†</td>
<td>47 ± 8‡</td>
</tr>
<tr>
<td>T cells</td>
<td>167 ± 18</td>
<td>44 ± 8‡</td>
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<tr>
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<tr>
<td>T cells</td>
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<td>52 ± 8*</td>
</tr>
<tr>
<td>B cells</td>
<td>82 ± 5</td>
<td>48 ± 6*</td>
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</tbody>
</table>

*Mice were adrenalectomized and maintained on corticosterone, 8 µg b.i.d. The PGE1-treated group received this compound (200 µg b.i.d.) for 14 days.

†Mean ± SEM x 16º.

‡Significantly different from controls (p < 0.005).

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**Fig. 1.** In vivo incorporation of 3HTdr by thymic and splenic cell suspensions, measured 1 and 4 hr after isotope administration. Treated animals received PGE1 for 2 days; the last injection of this compound was administered at the same time as the labeled thymidine. The isotope uptake in the two tissues was reduced by approximately 50% at both time intervals. Differences in each interval were statistically significant (minimal p value is less than 0.025).

**Fig. 2.** Mitogenic responses of the spleen cells to PHA, Con-A, and LPS in normal mice and in animals treated with PGE1, for 7- and 14-day intervals. The response to Con-A on day 14 was significantly greater than controls (p < 0.05).
prior to sacrifice. Those treated with cortisol received this steroid, 2.5 mg/mouse, 2 days prior to sacrifice, one group of animals received cortisol alone was reduced by 90%. Occurred in the thymus. The thymic cellularity in animals receiving cortisol alone was reduced by 90%. PGEI-treated mice who received a single injection of steroids 2 days prior to sacrifice showed an even greater reduction in thymic cellularity. In these animals, the number of thymocytes was decreased by 98%; this value was significantly reduced compared to that observed in animals treated with either cortisol or PGEI alone (p < 0.025). The additive effects of the two agents suggest that PGEI has activity against steroid-sensitive and steroid-resistant lymphoid populations in the thymus.

Cortisol caused a lesser decrease in splenic cellularity; the total number of spleen cells was reduced from 309 ± 22 × 10⁸ to 124 ± 23 × 10⁸ (p < 0.005). As shown in Table 5, this reduction was due to a decrease in both T and B cells. In accord with the effects observed in the thymus, additive activity was observed with the combination of PGEI and cortisol. Both types of lymphocytes were reduced by 84%.

**DISCUSSION**

Large quantities of parenteral PGEI can exert both immunosuppressive and immunoenhancing activities in vivo. The diversity in immune-modulating effects is illustrated in Table 6; in part, these may be due to such variables as animal species, quantity and duration of PGEI therapy, and the type of antigenic response evaluated. In general, maximum inhibitory activity appears to be directed at primary humoral immunity. By contrast, many secondary antibody reactions and cell-mediated responses are either unaffected or, in some instances, enhanced.

Clinical interest in PGEI, as a therapeutic agent has increased because of observations suggesting it can modify immunologically related diseases in three animal models. Prolonged administration significantly reduces adjuvant arthritis in rats. More recently, it has been shown that it can increase survival of NZB/W mice by lessening the intensity of the immune complex glomerulonephritis. Furthermore, it is able to decrease the nephritis observed in a murine model of acute serum sickness. As PGEI can affect many tissues, it has not been established whether these beneficial effects are due to immunosuppression or an antiinflammatory activity.

To date, the mechanisms responsible for the altered immune reactivity have not been fully defined. In particular, only preliminary data have been reported regarding the effects of pharmacologic quantities of PGEI on lymphocytes. Our studies indicate that this compound causes a selective reduction in lymphoid cells. The sites of lymphocyte depletion—the thymus, bone marrow and splenic B cell pool—are all characterized by rapid rates of cell renewal. By contrast, no changes were observed in the numbers of peripheral blood lymphocytes and splenic T cells. The majority of cells in these compartments have a long lifespan and a slow turnover rate.

To evaluate the basis for these lymphoid alterations, several correlative studies were performed. One parameter, the in vivo incorporation of ³HdR, was used as an indicator of intrinsic lymphopoiesis. By this criterion, PGEI significantly reduced isotope uptake, a finding suggesting that this agent inhibits new cell formation. These data correlate well with the effects of PGEI in vitro; it is a potent inhibitor of mitogen-induced lymphoproliferative responses. The specific mechanisms responsible for the in vivo phenome-
lymphocytes. No evidence that PGE1 exerted a significant lympholytic activity was found by histologic, cytologic, or cell viability criteria. These data do not exclude a cytotoxic activity but suggest that it is probably not a major factor. The lymphoid changes were not due to a corticosteroid effect, as similar changes were observed in adrenalectomized mice. Collectively, these findings suggest that the effect of PGE1 is primarily to reduce the rate of new cell formation. This would explain the preferential loss of short-lived lymphocytes; cells in these areas depend on the rapid rate of renewal to maintain homeostasis.

Residual lymphocytes from treated mice were not impaired in their in vitro responses to three polyclonal mitogens: PHA, Con-A, and LPS. The former two are stimuli for T cells, whereas LPS is a B-cell mitogen. These data suggest the in vitro effects are not the result of intrinsic cellular injuries. Rather, once cells are removed from an inhibitory environment, they can resume normal proliferative activity. This would account for prompt restoration of lymphoid cellularity following discontinuation of treatment. The modest increase in response to Con-A may be due to the proportional increase in splenic T cells, an alteration that results from the preferential loss of B lymphocytes.

Additional insight into the actions of PGE1 on lymphocytes was derived from studies that included corticosteroids in the treatment protocol. The effects of combined treatment were most apparent in the thymus. In the mouse, the majority of these lymphoid cells are readily lysed by corticosteroids. However, there is a minor population that is steroid-resistant; these thymocytes are distinct in that they are primarily responsible for most of the immunologic activity mediated by this organ. Based on assessments of thymic cellularity, cortisol and PGE1 appeared to have an additive effect; the cellular reduction in animals receiving both agents was significantly greater than that seen with either drug alone. Thus, these data suggest that PGE1 is capable of affecting both steroid-sensitive and -resistant thymocytes.

The mechanisms by which PGE1 promotes these lymphoid changes in vivo have not been fully defined. One likely effect is via stimulation of the enzyme adenylate cyclase; this, in turn, would increase intracellular concentrations of cAMP. This mechanism appears to account for the reduction in mitogen-stimulated lymphopoiesis in vitro when PGE1 is included in the culture media. Furthermore, serum PGE1 concentrations in treated mice are similar to those required for in vitro inhibition. It is noteworthy that, in the pharmacologic quantities employed in these studies, PGE1 appears to be metabolized slowly. Significant quantities of immunologically reactive compounds were present in the circulation 2 hr after administration. This contrasts with the very rapid disappearance of PGE1 in physiologic concentrations; it is largely inactivated after one passage through the lungs.

Additional studies showed that another adenylate-cyclase-stimulating compound, PGA1, caused similar lymphoid alterations. By contrast, PGF2α, which does not activate this enzyme system and is antagonistic to many of the effects of PGE1, did not reduce quantities of lymphocytes. Although these data suggest enzyme activation as a potential mode of action, it must be recognized that PGE1 has numerous and highly diversified pharmacologic properties. Thus, it is not possible to conclude that, in vivo, the lymphoid effects of PGE1 are mediated primarily by direct stimulation of the adenylate cyclase-cAMP system.

The other doses of PGE1 employed in the present studies are similar to those used in other investigations describing in vivo immune alterations and are far in excess of physiologic levels. As such, these data cannot be equated with the role of endogenously produced prostaglandins in vivo. Nevertheless, they indicate that pharmacologically, PGE1 is capable of altering lymphoid cellularity. By extension these changes may, in part, account for its immune-modulating effects. The net effect on immunity might result from either a decrease in effector cells or an alteration in cells regulating immune reactivity.

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REFERENCES

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