Impairment of Lymphocyte Sensitivity to Prostaglandin E2 in Cultures from Patients with Lymphoadenopathy Associated Syndrome*

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ABSTRACT

Prostaglandin E2 (PGE2) appears to have an immunosuppressive role in human immunodeficiency virus (HIV) infection. Therefore, the effect was studied of PGE2 pretreatment of T lymphocytes from patients with lymphadenopathy associated syndrome (LAS) on the expression of CD25 and CD71 as well as plaque forming cell (PFC) generation in pokeweed mitogen (PWM)-driven cultures. The PGE2-treated or untreated T lymphocytes were cultured with B cells and monocytes in the presence of PWM. Both CD25 and CD71 expression were assessed with an immunofluorescence technique; PFC generation was tested by hemolysis. Before exposure to PWM, LAS lymphocytes showed activation as evidenced by high CD25 and CD71 expression and PFC generation. Pretreatment by PGE2 did not inhibit expression of activation markers and PFC generation in LAS cultures, in contrast to what happened in control cultures. Thus, LAS lymphocytes are activated in vivo and are less sensitive to PGE2 inhibition than normal lymphocytes.

Introduction

Prostaglandins (PGs) have been intensively studied for their ability to upregulate or downregulate different functions of the immune system. Several studies indicate that PGs of the E series inhibit T cell mitogenesis and Interleukin-2 (IL-2) production, as well the synthesis of the IgM antibody. Accordingly, previous work from our laboratory has shown that PGE2 pretreatment of human peripheral lymphocytes inhibits B cell differentiation to Ig-secreting plasma cells in PWM as well as antigen-driven cultures. In these systems, T lymphocytes appear to be the primary target of PGE2. On the other hand, it was reported that PGs of the E series, as well as other agents capable of elevating intracellular cyclic adenosine monophosphate (cAMP), specifically increase IgE and IgG1 production.
Moreover PGs may be involved not only in normal immune responses, but also in human immunodeficiency virus (HIV)-induced immunological abnormalities. In fact, their presence in the seminal fluid may account for the immunosuppression associated with anal intercourse.\(^7\) In addition, their synthesis by monocytes is greatly increased in HIV infection.\(^{23}\) The hypothesis has been developed by us that high concentrations of PGE2 might affect lymphocyte response in the infectious disease caused by HIV.

The present authors were interested in investigating if the impairment of lymphocyte functions in HIV infection also involves an altered sensitivity to the immunoregulatory effect of PGE2. Thus, study was made of the response of lymphocytes from patients with lymphadenopathy associated syndrome (LAS) to exogenous PGE2 in pokeweed mitogen (PWM) stimulated cultures. It was shown that cultured T lymphocytes from patients with LAS were already activated and that treatment of the lymphocytes with PGE2 did not affect the expression of the two activation markers, CD25 and CD71, during the culture, in contrast to what happens when normal T cells were incubated with PWM and PGE2.\(^{25}\) Also B cell differentiation was unaffected by the prostaglandin, while PGE2-pretreated normal lymphocytes produced fewer plaque forming cells (PFC) than untreated cells.

**Materials and Methods**

**Patients**

Criteria for inclusion in this study were a positive HIV serologic reaction by enzyme linked immunosorbent assay (ELISA) and Western Blot and classification in the stage 3 of the Walter Reed system (LAS). All of the 15 patients enrolled in this study were intravenous drug abusers and had >400 CD4\(^+\) cells per mm\(^3\). Their age was 25 ± 3 (mean ± standard deviation, SD) years. Fifteen healthy volunteers of the same age and sex were also studied as controls.

**Preparation of Cells**

Mononuclear cells were separated from whole blood by using a standard Ficoll-Hypaque sedimentation technique.\(^3\) The T cell enrichment was performed by negative depletion using the panning method according to Mage et al.\(^{11}\) Briefly, Falcon polystyrene Petri plates, tissue culture grade, were coated at 4°C for 12 hours with the monoclonal antibody OKB22\(^*\) to prepare T cells by negative depletion. Two sequential rounds of panning were performed.

The recovered cells were >95 percent T lymphocytes as assessed by indirect immunofluorescence staining with CD3 monoclonal antibody.\(^*\)

**PGE2 Treatment of Lymphocytes**

The T cells were treated with PGE2 (lot 35F-08395)\(\dagger\) 10\(^{-4}\) M in Hank’s balanced salt solution (HBSS) for 30 min. at 25°C, washed with HBSS and resuspended just prior to culture.

**Cell Culture**

The T lymphocyte-depleted cells were recovered from panning plates by gentle scraping with a rubber policeman. These cells contained 80 to 90 percent B lymphocytes as assessed by positive immunofluorescence with an anti Ig antibody\(^*\) and 10 to 15 percent monocytes as assessed by non specific esterase staining. T- and B cell-enriched populations were recombined after T cell treatment

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\(^*\) Ortho Diagnostic System, Raritan, NJ.
\(^\dagger\) Sigma Diagnostics, St. Louis, MO.
in a 4:1 T:B ratio and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium,† supplemented with L-glutamine (2 mM), 100 U per ml penicillin, 100 mg per ml streptomycin and 15 percent heat inactivated fetal calf serum (FCS).‡

Cells (1 × 10⁶) were cultured in one ml volumes in 24-well plates§ in a humified atmosphere at 37°C with 5 percent CO₂ for seven days. Five μg of PWM were added to each well.

**MEASUREMENT OF SYNTHESIZED IMMUNOGLOBULINS**

The cells were harvested at day 7 and tested for the formation of plaque forming cells (PFC) according to Gronowicz.⁵ Cell viability was always >98 percent as tested by trypan blue exclusion test.

**CD25 AND CD71 MEASUREMENT**

Cultures were tested every day for CD25 and CD71 expression. An indirect immunofluorescence technique was employed, using respectively monoclonal antibodies OK26a and OKT9.*

**Results**

The mean percentage of CD25+ cells from normal donors increased from 2 percent at 0 time to 18, 25, and 35 percent at 24, 48, and 72 hours, respectively, after culture in the presence of PWM.

On the contrary, lymphocytes from patients with LAS showed a significantly higher percentage of CD25+ cells at 0 time which only slightly increased during culture (figure 1). When T lymphocytes from healthy donors were pretreated with PGE2 10⁻⁴ M, there was a significantly lower percentage of CD25+ cells at 24, 48, and 72 hours of culture, as compared to untreated cultures. On the contrary, PGE2 pretreatment of T lymphocytes from patients with LAS did not induce any inhibition. The kinetics of CD71 antigen expression was similar to that of CD25. In fact, as shown in figure 2, there was a significantly higher percentage of CD71+ cells from patients with LAS at 0 time, as compared to normal controls. This percentage did not increase further in the course of culture in the presence of PWM.

By contrast, the number of CD71+ lymphocytes from healthy donors was very low at 0 time and gradually increased during culture. Pretreatment of lymphocytes from normal donors with PGE2 significantly reduced the increase of CD71+ cells during the first three days of culture, while PGE2 was ineffective in this inhibition when LAS cells were cultured.

In figure 3 are shown the PFC mean ± SD of 0 and 7 day culture cells in the presence of PGE2 treated and untreated T lymphocytes from patients with LAS and from controls. Cells from donors with LAS contained a moderate number of PFC at 0 time, while control lymphocytes had very few. By contrast, at the end of culture in LAS cells the number of PFC was lower than that of control cultures. Moreover, PGE2 pretreatment of lymphocytes did not significantly reduce PFC in LAS cultures, contrary to what was observed in cultures obtained from normal donors in which there was a 60% inhibition.

**Discussion**

The results reported here demonstrate that HIV infection in LAS patients profoundly affects the lymphocyte response to PWM and also the sensitivity to the regulatory effect of PGE2.

Spontaneous activation of T cells is documented in these patients by the elevated percentages of CD25+ and CD71+ cells at 0 time. This observation is in agreement with the model proposed by

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† Grand Island Biological Co., Grand Island, NY.
‡ Elkay Lab. Products, Hampshire, UK.
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Miedema et al.\textsuperscript{13} According to this model, HIV infection leads to a gradually increasing CD4\textsuperscript{+} T cell turnover which is sufficient to maintain normal cell numbers for variable periods of time before CD4\textsuperscript{+} cell depletion occurs. Thus, the increased number of cells bearing the activation antigens CD25 and CD71 that we observed in subjects with LAS could be associated with an increased lymphocyte turnover. \textit{In vivo} activation can account also for the failure of PWM to increase the levels of these markers. In fact, the number of CD25\textsuperscript{+} and CD71\textsuperscript{+} T lymphocytes from patients with LAS remains nearly the same during the first three days of culture, in contrast with what happens with control lymphocytes. So, it may be conceivable that lymphocytes from LAS patients, activated only by HIV, become relatively less responsive to a mitogen like PWM. In addition, our results show clearly the escape of T lymphocytes of donors with LAS from PGE2 regulation, as evidenced by the lack of CD25 and CD71 inhibition induced by this prostaglandin during the culture.
The spontaneous immunoglobulin synthesis reported by several laboratories, including our own, is a prominent feature of seropositive subjects.\(^1,2,9\) Also, a poor in vitro reactivity to activating stimuli which paradoxically accompanies the spontaneous upregulation in B cell function has been well documented.\(^1,2,9\) Despite the fact that the initial PFC from LAS lymphocytes are higher than those produced by normal donors, after seven days of PWM culture our results indicate that the response to PWM of LAS lymphocytes is about 60 percent lower than that of normal donors. Moreover, the numbers of PFC observed after PGE2-treatment of LAS T cells remain quite the same, while the incubation of control lymphocytes with PGE2 is able to reduce drastically (>60 percent) the PFC generation. Our results indicate that an altered sensitivity to PGE2 may be an additional abnormality of lymphocytes from subjects with LAS.

These results may be relevant in view of the consideration that B and T cells have ample opportunity to contact PGE2-secreting cells like macro-
Lymphocyte sensitivity to prostaglandin E2 from LAS

Figure 3. Plaque forming cell (PFC) generation from treated and untreated lymphadenopathy associated syndrome lymphocytes. *Significant (p < 0.01) as compared with control cells receiving the same treatment by Student’s test. A 10⁻⁴ M prostaglandin E2 concentration has been incubated with 1 x 10⁶ cells for 30 min at 25°C. Washed cells were either cultured with pokeweed mitogen and evaluated after seven days for their ability to form PFC, or immediately tested for production of PFC, as described in Material and Methods section. Bars represent mean ± SD from 15 donors.

Phages, fibroblasts and follicular dendritic cells⁴,⁶,⁸,¹⁷ and that the status of these PG-synthesizing cells might dictate the character and the dimension of immune response.¹⁹ In HIV disease, macrophages are infected and some of their functions appear to be altered, including an increased production of PGE2.¹²,²⁰,²¹,²³,²⁴,²⁹ A continuous, prolonged exposure of lymphocytes to large amounts of PGE2 may render them unresponsive to this PG; the loss of its reasoning circulates immunoregulatory effect. The relative insensitivity of LAS lymphocytes to PGE2 is reminiscent of a similar phenomenon observed in neoplastic disease stage 1. In these patients an increase was observed of PGE2-resistance of lymphocytes as tested by the PG-induced inhibition of early E-rosette formation.¹⁴

Thus, it can be postulated by the authors that two serious diseases, HIV infection and cancer, in which large amounts of PGE2 are produced, share a common impairment of lymphocyte function: an escape from the immunoregulation exerted by PGE2.
References


