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Short Communication

Cyclooxygenase and lipoxygenase arachidonic acid metabolism by monocytes from human immune deficiency virus-infected drug users

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ABSTRACT

Prostaglandin E_2, thromboxane B_2 and leukotriene B_4 monocyte production have been determined in human immune deficiency virus (HIV)-infected drug users ($n = 36$) and healthy subjects ($n = 29$). Eicosanoids were extracted from the incubates using C_{18} solid-phase cartridges and determined by radioimmunoassay. An enhanced production of prostaglandin E_2 and thromboxane B_2 was detected in monocytes from HIV-positive drug users whether or not they had been previously stimulated with zymosan. Concomitant leukotriene B_4 increases were not observed. The results reported in this paper indicate that altered cyclooxygenase arachidonic acid metabolism in monocytes from HIV-infected drug users is associated with the severe cellular immunodysfunction characteristic of AIDS. In contrast, leukotriene B_4 does not seem to play a role in AIDS-associated immunosuppression.

INTRODUCTION

Prostaglandins of the E series are important arachidonic acid (AA) metabolites involved in the immune response [1]. Recent studies have demonstrated their immunosuppressor role in immunological functions. Furthermore, it is known that prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2) are the major cyclooxygenase metabolites of AA produced by human monocytes [2] and that these mononuclear cells can be infected by the human immunodeficiency virus (HIV) [3]. In line with previously reported work on increased PGE_2 and TXB_2 production by unstimulated

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monocytes from eight AIDS patients [4], that study has now been extended to a much larger population of HIV-infected drug users ($n = 36$, including the eight earlier reported cases). The *in vitro* effects of zymosan and indomethacin (a stimulant and inhibitor of AA metabolism, respectively) on monocyte PGE_2 and TXB_2 production from seven HIV infected drug users has also been evaluated.

As no data are available on leukotriene B_4 (LTB_4) production by monocytes from HIV-infected drug users, this lipoxygenase metabolite of AA was also included in this study. It is known that LTB_4 induces the T-lymphocyte suppressor cell population [5]. More recently it has been reported that LTB_4 induces lymphokine and monokine production by various T-cell types [6], although its effect on T-cell activation remains unclear [7].

Briefly, the aim of this further study was to validate the results obtained with eight patients and to expand the scope of the first study [4] to an additional number of new HIV-positive (HIV+) cases to strengthen the implications of altered arachidonic acid metabolism in patients suffering from AIDS.

EXPERIMENTAL

Chemicals

Tritiated PGE_2 (186 Ci/mmol), TXB_2 (210 Ci/mmol) and LTB_4 (150 Ci/mmol) were purchased from Amersham International (Amersham, UK). Methanol, acetonitrile and light petroleum (b.p. 40–60°C) were from Merck (Darmstadt, Germany). Methyl formate was from Fluka (Buchs, Switzerland). The C_{18} cartridges were obtained from Waters Assoc. (Milford, MA, USA).

Subjects

Arachidonic acid metabolism by monocytes was studied in a group of 36 HIV-infected drug users with or without AIDS, selected according to the Criteria of the Center for Disease Control Classification. In all cases HIV antibodies were assessed by two different enzyme-linked immunosorbent assays (Abbot-Elisa and Elavia-Institute Pasteur Tests) and confirmed by Western blotting. The absolute numbers of CD4+, the blastogenic response to mitogens *in vitro* and the response to antigens in delayed type hypersensitivity skin testing were evaluated in all patients. A group of 29 healthy volunteers (HIV−), both male and female, were studied as controls.

Monocyte incubations

Monocyte preparations from 36 HIV-infected drug users and 29 healthy controls were carried out as described elsewhere [8]. More than 95% of the adherent cells thus obtained were shown to be monocytes by May–Grünwald–Giemsa staining and positive reaction for esterase. The monocyte preparations were incubated for 24 h in RPMI-1540 medium supplemented with 1% fetal calf serum. Incubate supernantants were analysed for PGE_2, TXB_2 and LTB_4 as described in the following sections. Moreover, in monocyte preparations from seven of these HIV-infected drug users and healthy subjects, four monocyte cell preparation aliquots were incubated under the following conditions: basal, indomethacin (1 μg/ml), zymosan (160 μg/ml) and zymosan (160 μ/ml) + indomethacin (1 μg/ml); zymosan and indomethacin were added to study the synthesis and release of PGE_2 and TXB_2 in stimulated and non-stimulated cells, respectively.

Extraction of arachidonic acid metabolites

Tritiated standards (52 pg of PGE_2, 46 pg of TXB_2 and 58 pg of LTB_4) were added to the monocyte cell culture supernatants. The samples were acidified to pH 3 and directly processed through Sep-Pak C_{18} cartridges. After washing with 10 ml of water (pH 3.15) and 20 ml of light petroleum, the arachidonic acid metabolites were finally eluted with 8 ml of methyl formate. The residues, obtained after the evaporation of organic eluates to dryness, were resuspended in chromatographic or radioimmunoassay (RIA) buffer and subjected to high-performance liquid chromatography (HPLC) and/or RIA [7]. The recoveries were determined by scintillation counting.

HPLC purification

HPLC separation of the cyclooxygenase arachidonic acid metabolites PGE_2 and TXB_2 was carried out under isocratic conditions using 40 m*M* formic acid, pH 3.15, with triethylamine–acetonitrile (67:33, v/v) as the mobile phase at a flow-rate of 1.5 ml/min. A Spherisorb ODS-2, 10 μm, 25 × 0.46 cm I.D. column (Phase Separations, Deesire, UK) was used.

The biological samples were injected into a Kontron liquid chromatograph (Kontron Analytical, Zurich, Switzerland) and the appropriate fractions were collected at the elution time of the tritiated standards, established by a radioactivity detector (Ramona, Issomess, Straubenhardt, Germany) directly coupled to the HPLC system. After collection of the appropriate eluates, these 60-s fractions containing PGE_2 and TXB_2 were lyophilized and resuspended in RIA buffer.

RIA determinations

Resuspended extracts, either directly obtained from the Sep-Pak cartridges or previously purified by HPLC, were subjected to RIA quantification. TXB_2, PGE_2 and LTB_4 were determined in duplicate using specific antisera. The PGE_2 and TXB_2 antisera showed a cross-reactivity for TXB_2 and PGE_2 of less than 0.01 and 0.1%, respectively.

RESULTS

The C_{18} solid-phase extraction, HPLC and overall recoveries for PGE_2, TXB_2 and LTB_4 are given in Table I.

The immunoreactive peaks detected in the HPLC eluate fractions from the monocyte supernatants correspond to those eluates collected at the elution times of tritiated authentic standards of PGE_2 and TXB_2 (Fig. 1). No significant difference in the results was observed when the HPLC purification step was omitted. Linear correlations (r = 0.9899 and 0.97623, respectively) were observed for the RIA values of TXB_2 and PGE_2 obtained with and without prior HPLC purification.

Fig. 2 shows the TXB_2 and PGE_2 levels determined in monocyte preparations obtained from HIV infected drugs users (n = 7) and healthy volunteers (n = 7), in both instances incubated individually with indomethacin or zymosan as well as with a mixture of these two compounds. TXB_2 is the major cyclooxygenase AA metabolite synthesized by human monocytes under these conditions. A significant inhibitory effect on TXB_2 and PGE_2 production was observed in the presence of indomethacin

TABLE I

TXB_2, PGE_2 AND LTB_4 RECOVERIES (%) FROM MONOCYTE SUPERNATANTS EXTRACTED AND PURIFIED BY HPLC

Values are expressed as mean ± S.D. $n = 5$.

Metabolite	Recovery (%)		
	Extraction	HPLC	Extraction + HPLC
TXB_2	92.7 ± 3.8	64.6 ± 2.1	59.7 ± 1.7
PGE_2	93.7 ± 3.8	78.8 ± 4.7	72.4 ± 4.7
LTB_4	90.3 ± 2.3	80.6 ± 5.5	72.7 ± 6.1

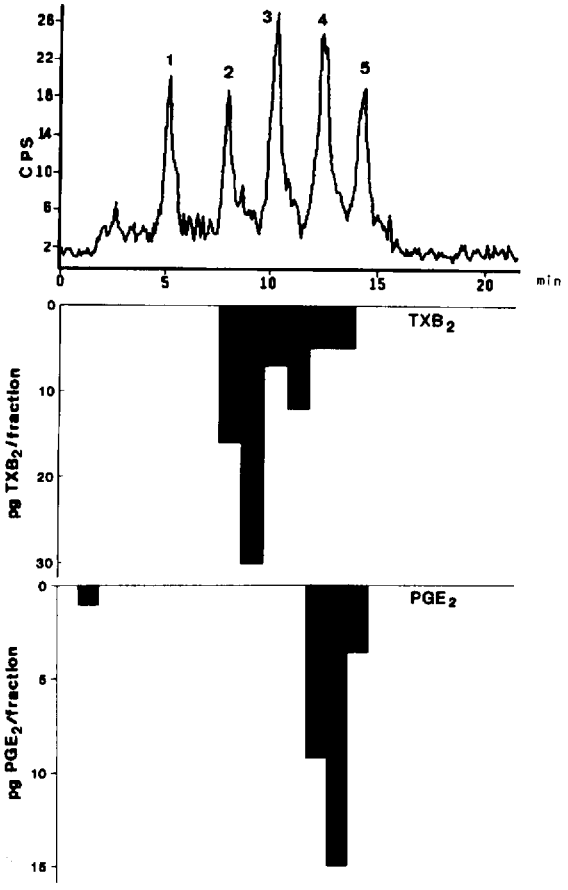

Fig. 1. On-line radiochromatogram of five prostanoids (top panel) and reconstructed immunochromatographic profiles of TXB_2 and PGE_2 (centre and lower panels) obtained by RIA of collected 60-s HPLC eluates of monocyte incubate supernatants. Mobile phase: 40 m*M* formic acid, pH 3.15, with triethylamine–acetonitrile (67:33, v/v), at a flow-rate of 1.5 ml/min. Peaks: 1 = 6-Keto-$PGF_{1\alpha}$; 2 = TXB_2; 3 = $PGF_{2\alpha}$; 4 = PGE_2; 5 = PGD_2.

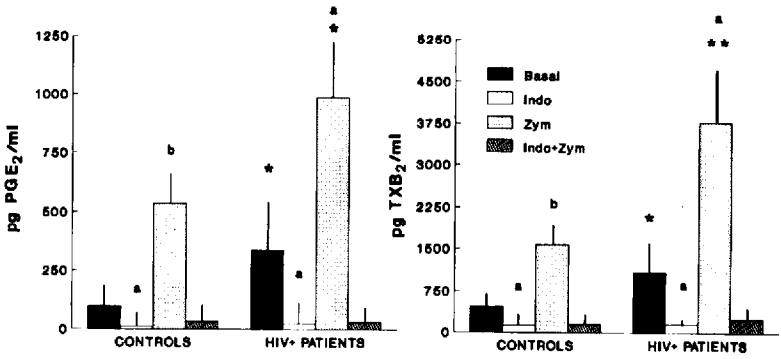

Fig. 2. PGE_2 (left) and TXB_2 (right) synthesized by monocytes from HIV-infected drug users ($n = 7$) and controls ($n = 7$), incubated under basal conditions and with the addition of indomethacin, zymosan or a mixture of both. ★: $p<0.1$; ★★: $p<0.05$ *versus* controls (Student's *t*-test); a: $p<0.05$; b: $p<0.005$ *versus* basal (*t*-paired test).

in both groups of subject ($p< 0.05$ *versus* basal). Also, a significant increase in TXB_2 and PGE_2 production was observed in the presence of zymosan ($p< 0.005$ in the control group and $p< 0.05$ in the HIV+ groups *versus* basal). In all instances prostanoid production in monocytes from HIV-infected drug users was higher than in the controls ($p< 0.1$, *versus* control), including monocytes incubated with zymosan ($p< 0.1$ for PGE_2 and $p< 0.05$ for TXB_2 *versus* controls).

Fig. 3 shows the basal production of PGE_2, TXB_2 and LTB_4 corresponding to HIV+ drug users ($n = 36$) and controls ($n = 29$). In agreement with earlier work [4], monocytes from these HIV-infected drug users also show an altered production of PGE_2 and TXB_2 ($p< 0.05$),but there were no variations in LTB_4, the major lipoxygenase AA metabolite synthesized by human monocytes.

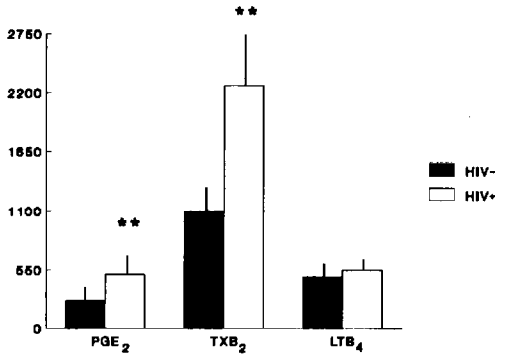

Fig. 3. Eicosanoid biosynthesis by monocytes from HIV+ drug users ($n = 36$) and healthy subjects ($n = 29$) with values including the seven cases described in Fig. 2. ★★: $p<0.005$ *versus* monocytes from HIV+ drug users (Student's *t*-test).

DISCUSSION

The immunochromatograms shown in Fig. 1 and the linear correlation between TXB_2 and PGE_2 values obtained pre- and post-HPLC, suggest that, contrary to recommended procedures [9], the HPLC purification of samples before RIA could be omitted in this instance, thus simplifying the experimental procedure. The direct RIA of biological extracts is often made difficult by lack of absolute specificity of the antibodies. This leads to well known instances of erroneous values as a result of cross-reactivity between structurally related compounds present in the samples [9]. However, in instances where the sample matrix is both relatively simple in its composition and free of analyte interferences, direct RIA could become a valid alternative. Nevertheless, for analytical reliability, the results of direct RIA should always be validated against the corresponding values from HPLC-purified fractions. These data, obtained as shown in Fig. 1 from a single pool of monocyte incubates, confirm that cyclooxygenase immunoreactivity is higher for TXB_2 than for PGE_2 and that the RIA quantitation is not affected by mutual interferences because of the extremely low cross-reactivity of the respective antibodies. This has been verified although TXB_2 shows considerable tailing, as shown by the corresponding immunochromatogram in Fig. 1 and the lower HPLC recovery values (Table I). This tailing effect, as previously discussed, is very difficult to counteract due to the dynamic equilibrium established between the open and closed forms of the oxane ring in TXB_2 [10,11]. However, the contribution of the approximately 5 pg TXB_2 present in each of the two 60-s fractions (collected between 12 and 14 min in Fig. 1) does not add more than 0.05 pg per fraction to the RIA values of PGE_2.

The pattern of prostanoid synthesis in human monocytes depends on the *in vitro* culture conditions. From this point of view, these results (see Fig. 2) agree with data reported previously [8,12]. The results in Fig. 2. show that monocytes from HIV-infected drug users (in basal or stimulated conditions) show a significant cyclooxygenase activation which is reflected in a concomitantly increased production of PGE_2 and TXB_2, the latter being the major cyclooxygenase AA metabolite. Although this tendency observed in a groups of seven patients is significant at $p = 0.1$, when the study was extended to a group of 36 patients and 29 controls (see Fig. 3), the observed differences were conclusive, showing a clear overproduction of prostanoids by monocytes from HIV-infected drug users with a higher statistical significance ($p < 0.05$).

PGE_2 has been accepted as an immunosupressing factor in cellular immunity [13]. However, the role of TXB_2 in immune regulation is unknown. TXB_2 seems to be involved in severe immunological responses such as organ rejection [14] and septicaemia [15]. With respect to the latter, it is known that the suppressed immune response of the patients with sepsis is also associated with enhanced TXB_2 plasma levels when compared to healthy subjects [15]. It is not known whether TXB_2 plays an immunosuppressive role in HIV-infected drug users. Further studies are necessary to elucidate whether this elevated TXB_2 production could contribute to increased immune deficiency in AIDS patients.

One interesting result of this study is that the LTB_4 production by monocytes is unaltered in the presence of HIV infection. These data seem to indicate a higher specific cyclooxygenase activity in monocytes from HIV-infected drug users, in con-

trast to the non-specific activation of human monocytes by several stimuli such as calcium ionophore or zymosan, which indistinctively potentiate both the cyclo and the lipoxygenase pathways. Thus, these results suggest that LTB_4 is not directly involved in the immunosuppressive mechanisms of AIDS, although it has been reported that, in addition to being a potent pro-inflammatory agent, LTB_4 can affect immunorregulatory functions [6].

In conclusion, an enhanced prostanoid production by monocytes from HIV-infected drug users has been confirmed. Moreover, these results also confirm that PGE_2 and TXB_2 are associated with HIV infection and suggest that altered cyclooxygenase AA metabolism may be one of the factors involved in the progressive and severe immunosuppression associated with AIDS.

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