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Indomethacin Modulation of Monocyte Cytokine Release following Pelvic Irradiation for Cancer

B. Petrini, G. Wolk, J. Wasserman, I. Vedin, Ö. Strannegård, H. Blomgren and P.-L. Esposti

Pelvic irradiation for urogenital cancer reduced monocyte release of tumour necrosis factor alpha (TNF-α). Addition of indomethacin to monocyte cultures increased TNF-α production after but not before irradiation. *E. coli* lipopolysaccharide (LPS) increased TNF-α release before as well as after radiation therapy and addition of indomethacin to LPS-stimulated monocytes further increased TNF-α production following radiotherapy. Spontaneous interleukin-1 (IL-1) release was increased in the cancer patients and was not significantly affected by radiation therapy. LPS increased IL-1 release before as well as after irradiation, but indomethacin did not further change IL-1 secretion. These findings suggest that prostaglandins differentially regulate TNF-α and IL-1 release. Administration of cyclo-oxygenase inhibitors during radiation therapy might increase TNF-α release *in vivo* and thereby enhance the host defence against tumours.
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INTRODUCTION

TUMOUR NECROSIS factor alpha (TNF-α) is a cytokine released by monocytes/macrophages when stimulated, e.g. by *Escherichia coli* lipopolysaccharide (LPS), and is generally considered to be a mediator of inflammation and cytotoxicity against tumour cells [1, 2]. Interleukin-1 (IL-1) is a cytokine with effects partly overlapping those of TNF-α [3], but also with important macrophage and lymphocyte activating properties [4]. It is known that TNF-α stimulates the release of prostaglandins and IL-1 [5] and that prostaglandin E_2 reduces LPS-triggered release of TNF-α [6, 7].

We have shown that radiation therapy for cancer may activate monocytes to prostaglandin-mediated suppression of immune reactivity [8] and that inhibitors of cyclo-oxygenase, which is important in biosynthesis of prostaglandins, could partly revert radiation-induced immunosuppression *in vitro* as determined by lymphocyte mitogen reactivity [9]. It is well known that cytokines play an important role in lymphocyte reactivity and proliferation [1–4]. Therefore we performed *in vitro* experiments to investigate whether radiation therapy affects cellular release of TNF-α and IL-1 and whether this process might be modulated by a cyclo-oxygenase inhibitor.

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MATERIALS AND METHODS

Patients and controls

14 patients, 13 men and 1 woman aged 59–76 years (mean 68) were examined. None of them had previously received radiation therapy or treatment with cytotoxic drugs. All men had moderately or poorly differentiated prostatic cancer without evidence of metastatic spread. The female patient had a poorly differentiated localised bladder cancer of urothelial differentiation. 10 female and 6 male laboratory staff members, aged 22–57 years (mean 40) served as controls for intertest variability.

Radiation therapy

All the patients received 8 MV radiation therapy from a linear accelerator using an anterior open beam and two oblique wedge filter beams from the back. The 100% isodose included most of the false pelvis. Radiation therapy was given 5 days a week with a cycle dose of 1.8 Gy, up to 63 Gy (prostate cancer) and 64.4 Gy (bladder cancer).

Blood sampling

Blood was drawn from patients at the start and at completion of irradiation. Healthy controls were examined twice with an interval of approximately 6–12 weeks.

Isolation of monocytes

The procedure, which is a slight modification of that described by Boyum [10], was detailed previously [11]. In short, EDTA containing blood was sedimented with gelatine for 1 h at 37°C. The supernatant, containing leucocytes, was layered over Nycodenz (Nyegaard, Oslo, Norway) and centrifuged at 600 *g* for 15 min. The monocyte-rich interphase was washed at 400 *g* and 200 *g* for 10 min in phosphate buffered saline without Mg^{2+} or Ca^{2+} and in RPMI 1640 medium at 150 *g* for 10 min. The preparations contained over 95% viable monocytes.

Culture conditions

Purified monocytes, 1×10^6 per ml of RPMI medium with 10% fetal calf serum were incubated for 22 h in a humidified CO_2- air atmosphere as follows: medium alone, 10^{-5} mol/l indomethacin (Merck, Sharp & Dohme), 10 μg/ml of LPS (Sigma L-3137), and both indomethacin and LPS. Indomethacin was first dissolved in absolute ethanol. The final ethanol concentration was 1/1000 in cultures. This concentration did not affect cytokine release (data not shown). Supernatants were kept at −70°C before analysis.

Determination of TNF-α

Kits for radioimmunoassays of TNF-α were purchased from Medgenix, 1160 Brussels, Belgium.

Determination of IL-1 β

An enzyme-linked immunosorbent assay from Cistron, manufactured by Eurogenetics, Tessenderlo, Belgium, was used.

Statistical analyses

Student's *t* test for uncorrelated and correlated means was used to calculate statistical differences between groups and before/after treatment, respectively.

RESULTS

Reproducibility of tests for cytokine release

Figure 1 shows that differences between values of the first and second tests in healthy controls were non-significant for both TNF-α and IL-1.

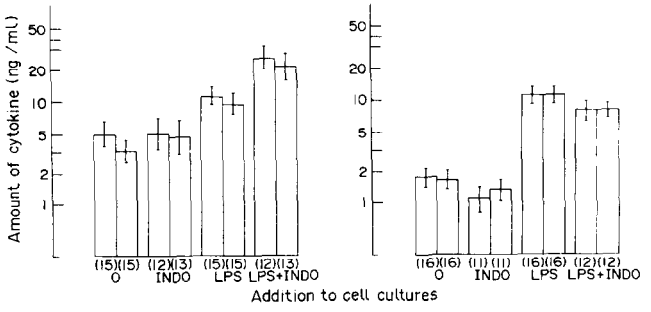

Fig. 1. Release of TNF-α (left) and IL-1 (right) in monocyte cultures from healthy controls tested on two occasions, 6–12 weeks apart. Geometrical means (S.E.) are given. Numbers of patients are given in parentheses in each column. Hatched columns denote tests following radiation therapy. Additions to cell cultures: 0 = medium only, INDO = indomethacin 10^{-5} mol/l, LPS = lipopolysaccharide (10 μg/ml).

Table 1. Monocyte production of cytokines in non-irradiated cancer patients (n = 14) and healthy controls (n = 15–16)

Addition to culture	TNF-α	IL-1
0		
Patients	3.82 (0.27)	3.64 (0.13)*
Controls	3.67 (0.14)	3.25 (0.11)
Indomethacin		
Patients	3.81 (0.19)	3.31 (0.19)
Controls	3.68 (0.16)	3.04 (0.14)
LPS		
Patients	4.18 (0.12)	4.18 (0.09)
Controls	4.24 (0.09)	4.04 (0.08)
LPS + indomethacin		
Patients	4.31 (0.17)	4.03 (0.12)
Controls	4.40 (0.11)	3.92 (0.10)

$\log_{10}$ mean (S.E.) of pg/ml.

* Significant difference between patients and controls ($P = < 0.02$, *t* test). Other such differences are non-significant.

Cytokine release in healthy controls and untreated patients with urogenital cancer

The spontaneous release of TNF-α was similar in patients and controls (Table 1). Spontaneous IL-1 release on the other hand was higher in patients than in controls ($P < 0.02$). Monocyte secretion of TNF-α as well as IL-1 in the presence of indomethacin and LPS, respectively, was similar in patient and control groups. Indomethacin alone did not significantly affect cytokine release in either group. LPS however enhanced release of both cytokines in patients as well as controls ($P < 0.0005$). Addition of indomethacin to LPS-treated cultures further increased TNF-α release in controls ($P < 0.005$) but not in the patients. LPS-induced IL-1 release was unaffected by indomethacin in both groups.

Cytokine release following pelvic irradiation

As shown in Fig. 2 irradiation significantly reduced spontaneous TNF-α secretion ($P < 0.05$) whereas addition of indomethacin reverted this effect ($P < 0.025$). LPS sharply increased TNF-α secretion before as well as after radiotherapy ($P < 0.0001$). There was no significant difference in values from LPS-treated cultures examined before and after therapy. Addition of indomethacin to LPS treated cultures had no effect

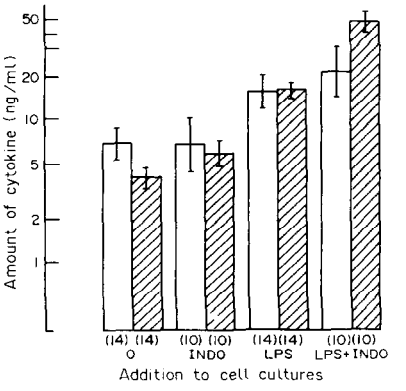

Fig. 2. Release of TNF-α in monocyte cultures from patients given pelvic irradiation.

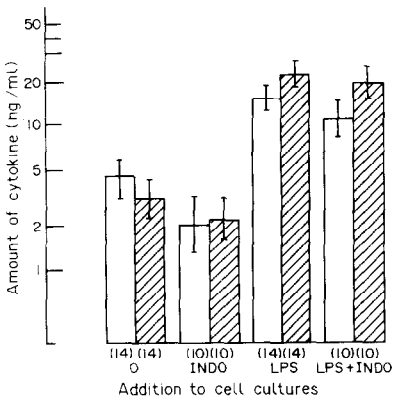

Fig. 3. Release of IL-1 in monocyte cultures from patients given pelvic irradiation.

before but further raised TNF-levels after therapy ($P < 0.0005$). The difference between TNF-values from such cultures in patients examined before and after irradiation was statistically significant ($P < 0.025$).

As shown in Fig. 3 irradiation did not change spontaneous release of IL-1 and addition of indomethacin had no effect. LPS increased IL-1 release before as well as after radiotherapy ($P < 0.0005$). Addition of indomethacin to LPS treated cultures did not change IL-1 levels before or after radiation therapy.

DISCUSSION

In the present study we found no significant differences regarding spontaneous release of TNF-α by monocytes from cancer patients and controls. By contrast, the release of IL-1 was significantly elevated in the patients. The latter finding suggests that monocytes in non-irradiated patients with pelvic cancer are to some extent activated *in vitro*. This suggestion would agree with previous findings of enhanced spontaneous release of IL-1 by monocytes from patients with hepatocellular cancer [12] and also with the earlier demonstration of increased serum levels of neopterin, another substance produced by activated monocytes/macrophages, in patients with prostatic cancer [13]. We found that TNF-α and IL-1 could be similarly stimulated by LPS in patients and controls, suggesting that there was no exhaustion with associated functional impairment of the monocytes in the patients. In other studies LPS-induced production of IL-1 has been found to be decreased in patients with malignant disease [12, 14, 15] the degree of depression correlating with tumour burden. The finding of normal LPS-induced IL-1 and TNF-α release in our cancer patients could thus probably reflect the fact that the tumour burden was small, without evidence of metastases in any patient.

Radiation therapy clearly suppressed the spontaneous release of TNF-α. This would be an expected finding since our previous studies have shown that radiation can induce prostaglandin mediated suppression of immune reactivity [8] and prostaglandin E_2 is inhibitory to TNF-α secretion [6, 7]. A further indication that the suppressive effect of irradiation on TNF-α secretion was indeed mediated by prostaglandins was our finding that the ability to release TNF-α could be restored by the cyclo-oxygenase inhibitor indomethacin.

LPS, which is a potent stimulator of TNF-α secretion [1], augmented TNF-α release by monocytes obtained before as well as after radiation therapy. Further treatment with indomethacin, however, resulted in pronounced stimulation of TNF-α secretion in cells obtained postirradiation only. Again, these findings would agree with the suggestion that the suppressive effect of irradiation on TNF-α was mediated by prostaglandins.

The suppressive effect of irradiation on TNF-α secretion may have clinical implications. TNF-α can cause destruction of tumour cells *in vitro* [16] as well as *in vivo* [17] and would therefore be supposed to play a beneficial role in cancer. However, in addition to its antitumoral effects TNF-α has strong immunomodulatory effects [3] and induces deleterious effects of endotoxin shock and chronic cachexia [18–20]. Therefore, irradiation induced suppression of TNF-α secretion cannot, *a priori*, be supposed to be disadvantageous to the treated patient. However, the finding that indomethacin can restore TNF-α responsiveness in irradiated patients deserves attention, in particular since cyclo-oxygenase inhibitors can restore also other types of immune functions in these patients [9, 21].

In contrast to the findings obtained with TNF-α, irradiation did not significantly suppress IL-1 secretion and we did not find any evidence of prostaglandin-mediated suppression of the production of this cytokine. Although it has been demonstrated that prostaglandins may suppress IL-1 [22, 23] as well as TNF-α formation [6, 7] our results suggest that separate mechanisms regulate TNF-α and IL-1 release. This suggestion would be compatible with the recent finding that different subsets of human blood monocytes seem to produce TNF-α and IL-1β [24]. The clinical implications in tumour disease of a differential regulation of IL-1 and TNF-α release are not clear. However, a preferential stimulation of TNF-α production after indomethacin treatment may, as discussed above, possibly have beneficial effects in the cancer patient. Moreover, since indomethacin also enhances IFN-γ secretion (unpublished results) and since IFN and TNF-α may act synergistically on tumour cell killing [25], treatment with indomethacin may possibly result in enhanced tumouricidal effect. On the other hand, the relative lack of influence of indomethacin on IL-1 production might be advantageous due to the inflammatory properties of the latter substance.

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Oral Contraceptive Use and the Risk of Ovarian Cancer: an Italian Case-Control Study

Fabio Parazzini, Carlo La Vecchia, Eva Negri, Luca Bocciolone, Luigi Fedele and Silvia Franceschi

The association between oral contraceptive (OC) use and the risk of ovarian cancer was analysed in a case-control study, conducted between 1985 and 1989 on 505 epithelial ovarian cancer cases under 60 years of age, and 1375 controls in hospitals for a spectrum of acute conditions, not gynaecological, hormonal or neoplastic, apparently unrelated to OC use. 41 (8.1%) women with epithelial ovarian cancer and 192 (14.0%) controls reported OC use. The multivariate relative risk (RR) for ever use was 0.7 (95% confidence interval (CI) = 0.5–1.0). The risk decreased with duration of use: compared with never users the multivariate RRs were 0.9 and 0.5 respectively for less than 2 years and 2 years or more users (χ^2_1 trend = 6.17, P = 0.01). The risk of ovarian cancer decreased with recency and latency of use: the estimated RR were 0.5 and 0.9 in women reporting last OC use less than 10 or 10 years or more from the diagnosis of the disease, and 0.6 and 0.8 in those reporting first OC use less than 10 or 15 or more years before. The protective effect of OC was consistent in separate strata of selected covariates, including parity and other major known or suspected risk factors for ovarian cancer. There was some indication that the protection declines with advancing age, but the risk estimates were similar in premenopause and postmenopause.
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INTRODUCTION

SINCE THE late 1970s, several epidemiological investigations have shown that oral contraceptive (OC) use lowers the risk of ovarian cancer [1–19].

An overview of published case-control studies indicated that the protection amounts to about 40% for women who used OC compared with never users and that this protection increases with duration of use. The reduced risk seems to persist in the medium period after pill use (at least 10 years), but the data did not provide definite relative risk estimates for longer periods or in relation to latency or recency of use, and for the potential interaction between pill use and the main risk factors for