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ORIGINAL ARTICLE

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Macrophage inflammatory protein (MIP)1α and MIP1β differentially regulate release of inflammatory cytokines and generation of tumoricidal monocytes in malignancy

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Abstract The C–C chemokines, macrophage inflammatory protein (MIP)1α and MIP1β are potent chemoattractants for the monocytes, which form an important component of the stroma of tumor tissue and may regulate tumor growth and associated inflammation. We examined the role of MIP1α and MIP1β in inducing the release of inflammatory cytokines and the generation of tumoricidal monocytes from the peripheral blood monocytes (PBM) of healthy women and patients with carcinoma of breast (CaBr). Interleukin-1 (IL-1) and tumor necrosis factor (TNF) α release by the PBM was markedly stimulated by MIP1α in CaBr patients, but only marginally so in healthy women. In contrast, MIP1β stimulated the release of these cytokines by the PBM of healthy women, but failed to do so in CaBr patients. MIP1α, but not MIP1β, synergized with LPS in inducing the release of IL-1 from the PBM of both healthy women and CaBr patients. Both MIP1α and MIP1β augmented respiratory bursts in PBM and generated tumoricidal PBM that killed T24 cells, MIP1α being more effective in CaBr patients and MIP1β in healthy women. IFN-γ co-stimulated and IL-4 suppressed MIP1α and β-induced cytotoxicity in PBM. The synergy of IFN-γ was more marked with MIP1α than with MIP1β. The differential effects of MIP1α and MIP1β on the PBM of healthy women and CaBr patients co-related with the levels of expression of CCR1 and CCR5 in these monocytes. The expression of CCR5 was higher than that of CCR1 in the PBM of healthy women and the PBM of the CaBr patients showed overexpression of CCR1 and downregulation of CCR5.

Keywords MIP1α · MIP1β · IL-1 · TNFα · Monocytes

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Introduction

The monocytes and tissue-associated macrophages play a major role in an acute or chronic inflammation, such as soft tissue trauma, infection, autoimmune disorder, and cancer, through the secretion of chemokines and cytokines. In tumor tissues, these cells form an important component of the stroma, but their role in regulating tumor growth is not clear. The tumor infiltrating monocytes (TIM) and peripheral blood monocytes (PBM) display varied levels of cytotoxicity that can be related to their local microenvironment. Tumor-growth-associated inflammation is dependent on the activation of endothelium and the infiltration of leukocytes [26, 31] induced by the C–C and C–X–C chemokines generated by the activated endothelium. The early response cytokines Interleukin-1 (IL-1) and tumor necrosis factor (TNF) α determine the adhesion to the endothelium and transendothelial migration of the leukocyte [34]. The detection of C–C chemokines, in particular monocyte chemo-attractant protein-1 (MCP-1), in various tumor tissues [44] and their role in determining the level of TIM [25] suggest that the C–C chemokines have a role in regulating the tumor growth. MCP-1 and macrophage inflammatory protein (MIP)1α and MIP1β, activate respiratory bursts in monocytes [16, 46]. MCP-1 has also been shown to induce tumoricidal macrophages in synergy with LPS [38]. On the other hand, tumor growth and metastasis can be promoted by MCP-1 [1, 42]. The MCP-1-driven TH2 responses, the impairment of IL-12

production, and the activation of gelatinase- and urokinase-type plasminogen activator [8, 32] may be associated with the chemokine-induced augmented tumor growth and metastasis. MIP1α and MIP1β, in contrast to the effect of MCP-1, drive TH-1 responses [35, 39]. The regulation of monocyte migration, the induction of respiratory bursts in monocytes/macrophages, and the driving of TH-1 responses by MIP1α and MIP1β suggest that these chemokines may control the host's antitumor responses. In malignancy, a defective systemic immunity along with TH-1/TH-2 imbalance is often observed [5, 6]. The monocytes from cancer patients also have defects in their ability to respond to chemo-attractants [9]. In this paper, we examined the role of MIP1α and MIP1β in inducing inflammatory cytokines and tumoricidal monocytes in breast cancer patients.

Materials and methods

Patients

Eight patients with adenocarcinoma of breasts (CaBr patients) (clinical stages II and III), with ages ranging between 35 and 55, attending the Chittaranjan Cancer Hospital, Calcutta, India, were included in the study and the prescribed ethical norms of the institute were strictly adhered to. Blood samples were collected from the patients before they underwent any treatment. Seven age-matched healthy women served as the control group. All the patients and healthy women were from a middle socio-economic background, non-smokers, hepatitis B and HIV negative.

Peripheral blood lymphocytes (PBL) and PBM

The PBL and PBM from the blood samples of healthy women and CaBr patients were isolated by Histopaque (Sigma Chemicals Company, St. Louis, MO, USA) density gradient centrifugation and adherence. As was confirmed by non-specific esterases staining, more than 96% of the adherent cells were monocytes.

Culture medium

RPM1-1640 supplemented with 10% fetal bovine serum (LPS-free), 100 U/ml penicillin, and 10 µg/ml streptomycin (CM) was used throughout this study. The materials were procured from GIBCO, BRL, Gaithersburg, MD, USA.

Cell lines

T24 (human urinary bladder transitional cell carcinoma) and L929 (mouse fibroblastoid cell line) cells obtained from the National Facility for Animal tissue and Cell Culture, Pune, India, were cultured in CM and used as targets in cytotoxicity assay and TNF bioassay, respectively.

Chemokines, cytokines, and antibodies

The human recombinant MIP1α, MIP1β, and IL-4 were obtained as a gift from NCI, Frederick, MD, USA. Human recombinant IFN-γ, rabbit anti-human MIP1α, MIP1β, IL-1, IFN-γ, and IL-4 antibodies were purchased from (Biogen Research Corporation, Cambridge MA, USA) Peprotech, EC Ltd., London, UK and Pharmingen, Singapore.

In-vitro culture of PBM and PBL

The PBM and PBL were suspended in CM and plated into the wells of 96 well microculture plates at desired concentrations. The cells were cultured for 18 h (unless otherwise specified) at 37°C in 5% CO_2 atmosphere in the presence or absence of the C–C chemokines and cytokines.

Cytokine assays

The IL-1 bioactivity in the monocyte culture supernatants (CS) was determined by assaying the thymocyte co-mitogenic activity of the CS [28]. The thymocytes isolated from Balb/c mice were cultured for 72 h in CM in the presence of ConA (2.5 µg/ml) and aliquots of serially diluted monocyte CS. The monocyte CS added with anti-IL-1 antibody (diluted 1:100) and the monocyte-free CM was used as controls. The proliferation of the thymocytes was measured by MTT (Sigma) colorimetric assay [29].

The tumor necrosis factor bioactivity in the CS of PBM was assayed by measuring the CS-induced death of actinomycin D (Sigma)-treated L929 cells, as described earlier [18]. In brief, the L929 cells suspended in the CM were plated (5×10^5 cells/well) in 96 well flat-bottomed microtitre plates. Actinomycin D was added to the cells to a final concentration of 1 mg/ml. The cells were incubated for 18 h in the presence or absence of test CS. MTT colorimetric assay was done and the percentage dead cells (% DC) were determined from the OD values of the culture-supernatant-treated and -untreated L929 cells.

Microcytotoxicity assay

The cytotoxicity of PBM against the T24 cells was measured by co-culturing the PBM with T24 cells at an effector : target ratio of 1:40 for four hours at 37°C in 5% CO_2 atmosphere in a 96 well microculture plate following a method published earlier [13]. The LDH

released from the target cells in the culture supernatant was measured using a commercially available kit (Boeringer Mannheim, Indianapolis, IN, USA). The results were expressed as percent cytotoxicity, determined by taking into consideration the LDH released by the effector cell and target cell controls.

Cytotoxicity (%) = [{(OD of effector–target cell mix-OD of effector cell control)-OD of low control}/OD of high control-OD of low control]×100.

The low control or spontaneous LDH release was measured from the wells containing 1×10^4 T24 cells in assay medium. The high control (maximum LDH release) was determined by plating 1×10^4 cells in each of the triplicate wells containing 100 µl of 1% Triton X-100. The effector cell control was determined by plating PBM (1×10^5 cells/well) in triplicate wells and incubating the cells alone in the assay medium.

Assay for superoxide anion production

The production of superoxide anion (O_2^-) by the PBM was measured by assaying the Superoxide dismutase (SOD) (Sigma) inhibitable reduction of Ferricytochrome C (Sigma) by a technique modified from that described by Pike and Mizel [33]. The PBM suspended in CM were plated in a 96 well microtitre plate (10^6 cells/well) and cultured in the presence or absence of the chemokines for 18 h at 37°C in 5% CO_2 atmosphere. The cells were treated with PMA (0.5 µg/ml) as control. The cells were washed and the monolayer was cultured with Krebs ringer phosphate dextrose (KRPD) medium containing 80 µM of Ferricytochrome C in the presence or absence of SOD (100 µg/ml) for 60 min at 37°C. The change in the absorbance of the wells was measured at 550 nm. The rate of O_2^- production was expressed as a unit of nmol $O_2^-/10^6$ cells/60 min.

Results

MIP1α and MIP1β differentially modulate the release of IL-1 from the PBM of healthy women and CaBr patients

The PBM of healthy women and CaBr patients were cultured in the presence or absence of different doses (10–50 ng/ml) of MIP1α or MIP1β for 18 h. The culture supernatants were collected and the IL-1 bioactivity of the CS was determined. As shown in Fig. 1, MIP1α at 20- and 50-ng/ml doses stimulated IL-1 release from the PBM of CaBr patients, whereas MIP1β in similar doses stimulated IL-1 release from the PBM of healthy women. A marginal stimulation of IL-1 release from the PBM of healthy women was obtained with 10 and 20 ng/ml of MIP1α. The MIP1β failed to stimulate IL-1 release from the PBM of CaBr patients. The inhibition of the thymocyte co-mitogenesis following the addition of 1:100 diluted anti-IL-1 antibodies in the CS indicated specificity of the IL-1 bioactivity in the CS.

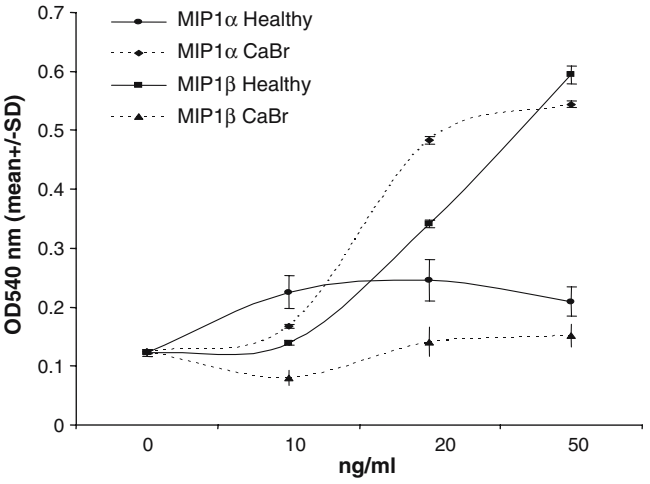

Fig. 1 MIP1α and MIP1β differentially modulate IL-1 release from the PBM of healthy women and CaBr patients. The PBM of seven healthy women and eight CaBr patients were cultured with (0–50-ng/ml) MIP1α and MIP1β for 18 h. The IL-1 released in the culture supernatants was determined by assaying thymocyte co-mitogenic activity of the culture supernatants. The data (mean ± SD) is representative of seven independent setsof experiments in the case of healthy women and eight sets of independent experiments in the case of CaBr patients

Synergy of MIP1α with LPS in inducing IL-1 release from the PBM of CaBr patients

The PBM of healthy women and CaBr patients were treated with LPS (1 µg/ml) alone, MIP1α (10, 20, and 50 ng/ml) alone, MIP1β (10, 20, and 50 ng/ml) alone and with combined doses of MIP1α and LPS or MIP1β and LPS for 18 h. Figure 2a shows that the LPS-induced IL-1 release by the PBM is less in CaBr patients than in healthy women. As shown earlier, MIP1α alone at all doses induced IL-1 release by the PBM of healthy women and CaBr patients. The MIP1α at 10 ng/ml was co-stimulatory with LPS and markedly enhanced IL-1 release by the PBM of healthy women and CaBr patients (Fig. 2a). In contrast, no synergy of MIP1β with LPS was observed in inducing IL-1 release from the PBM of either healthy women or CaBr patients (Fig. 2b).

MIP1α and MIP1β differentially modulate TNFα and superoxide anion release by the PBM of healthy women and CaBr patients

The PBM of the healthy women and CaBr patients were treated with 10, 20, and 50 ng/ml of MIP1α and MIP1β for 18 h at 37°C in 5% CO_2 atmosphere. The cells were washed and further cultured in CM for 18 h and the TNFα bioactivity in the CS was determined. The PBM of CaBr patients secreted significantly less ($P<0.01$) amount of TNFα as compared to that secreted by the PBM of healthy women (Fig. 3). The MIP1α enhanced TNFα release highly significantly ($P<0.001$) by the PBM of CaBr patients and less potently by the cells of

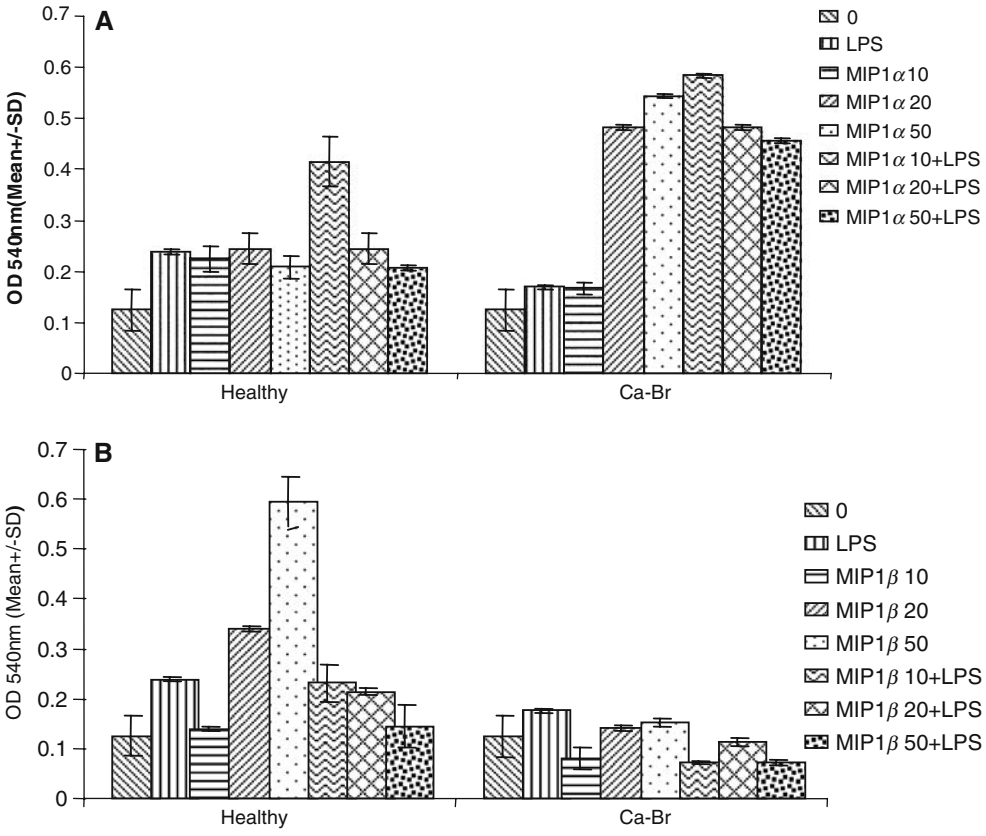

Fig. 2 Synergy of MIP1α and MIP1β with LPS in inducing the IL-1 release from the PBM of healthy women and CaBr patients. **a** MIP1α synergies with LPS in inducing IL-1 release. **b** MIP1β does not synergize with LPS in inducing IL-1 release. The PBM from seven healthy women and eight CaBr patients were cultured in the presence or absence of LPS (1–μg/ml) alone, 10–50–ng/ml of MIP1α or MIP1β alone and with combined doses of MIP1α and LPS or MIP1β and LPS for 18 h. The IL-1 released in the culture supernatants was determined by assaying thymocyte co-mitogenic activity of the culture supernatants. The data (mean ± SD) are representative of seven and eight sets of independent experiments for healthy women and CaBr patients, respectively

healthy women. In contrast, the MIP1β significantly ($P < 0.001$) enhanced TNFα release by the PBM of healthy women, but not so in CaBr patients (Fig. 3).

To determine the superoxide anion release, the chemokine-treated cells were washed and further cultured in KRPD medium containing Ferricytochrome C in the presence or absence of SOD (100 μg/ml) for 1 h at 37°C.

Figure 4 shows that the O_2^- production by the PBM of CaBr patients was deficient as compared with that by the PBM of healthy women. MIP1α augmented O_2^- release from the PBM of both healthy women and CaBr patients in a dose-dependent manner, but the stimulatory effect of MIP1α was more marked in CaBr patients. MIP1β in all doses (10–50 ng/ml) significantly enhanced ($P < 0.01$) O_2^- release by the PBM of healthy women. Only a marginal stimulation of O_2^- release in CaBr patients was observed following the treatment of the PBM with 50-ng/ml MIP1β (Fig. 4).

MIP1α and MIP1β stimulate tumor target killing by the PBM of healthy women and CaBr patients

The PBM of healthy women and CaBr patients were treated with or without 10–50 ng/ml of MIP1α or MIP1β for 18 h. The cells were then washed and co-cultured with T24 cells to determine their cytotoxicity against the T24 cells. The results of microcytotoxicity assays revealed that the PBM of both healthy women and CaBr patients could kill T24 cells (Fig. 5). MIP1α and β stimulated cytotoxicity of the PBM of both healthy women and CaBr patients in a dose-dependent manner. However, the cytotoxicity of the PBM induced by MIP1α was more marked in CaBr patients and that by MIP1β in healthy women. In a few experiments, the PBM of healthy women and CaBr patients were treated with MIP1α (50 ng/ml) plus anti-MIP1α antibodies (diluted 1:100) and MIP1β (50 ng/ml) plus anti-MIP1β antibodies (1:100). The treatment with the antibodies partially blocked (60–70%) the chemokine-induced cytotoxicity of the PBM.

IFN-γ co-stimulates and IL-4 inhibits the MIP1α- and MIP1β-induced tumoricidal activities of PBM

The PBM of healthy women and CaBr patients were cultured in the presence or absence of 50 ng/ml of MIP1α or MIP1β alone, 100 U/ml of IFN-γ alone or 200 U/ml of IL-4 alone and MIP1α (50 ng/ml) or MIP1β (50 ng/ml) along with IFN-γ (100 U/ml) or IL-4 (200 U/ml) for 18 h at 37°C in 5% CO_2 atmosphere. The cytotoxicity of the monocytes against the T24 cells and the release of O_2^- and TNFα from the PBM were measured. Figure 6a shows that IFN-γ alone enhanced the tumor killing by the PBM of both healthy women and CaBr patients. With MIP1α and MIP1β, the IFN-γ produced synergistic effect and significantly augmented the cytotoxicity of the PBM of healthy women and CaBr

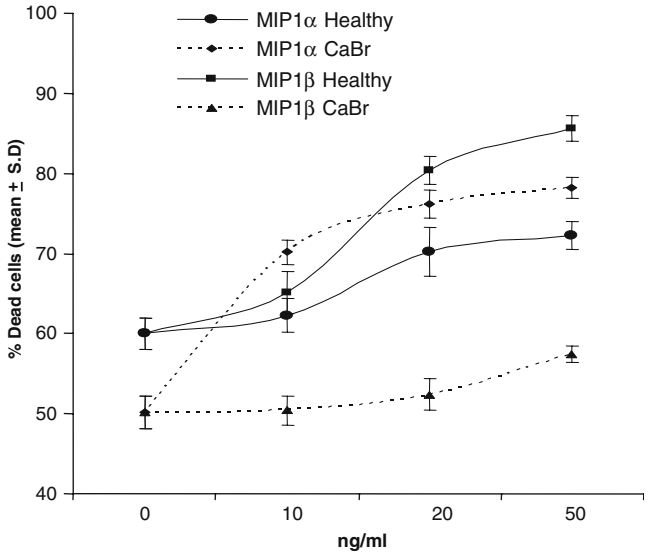

Fig. 3 Differential induction of TNFα release from the PBM of healthy women and CaBr patients by MIP1α and MIP1β. The PBM of six healthy women and six CaBr patients were cultured with or without 10–50 ng/ml of MIP1α or MIP1β for 18 h. The TNF bioactivity in the culture supernatants was determined by measuring the culture supernatant-induced death of Actinomycin-D-treated L929 cells in 18-h culture by MTT colorimetric assay. The data are mean ± SD of six sets of separate experiments for healthy women and CaBr patients separately

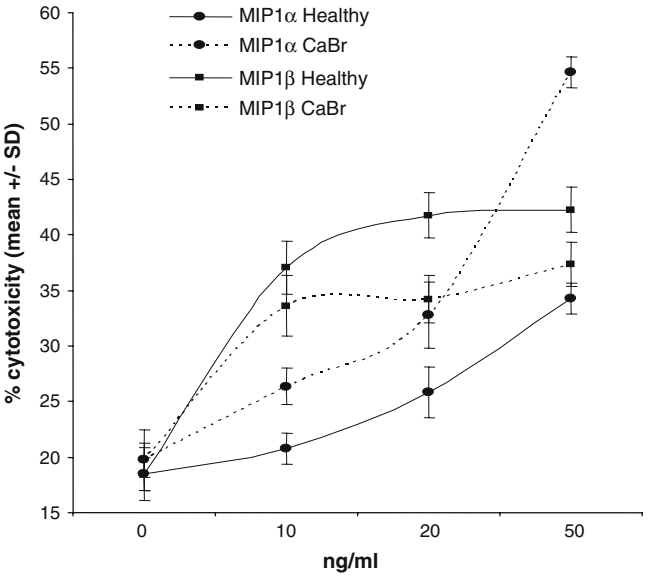

Fig. 5 MIP1α and MIP1β induce tumor killing by the PBM of healthy women and CaBr patients. The PBM of six healthy women and six CaBr patients were cultured in the presence or absence of 10–50-ng/ml MIP1α or MIP1β for 18 h. The T24 tumor target cells were then co-cultured with PBM at an E:T ratio of 20:1 for 4 h and the cytotoxicity of the PBM against the T24 cells was determined by assaying LDH released specifically by the T24 target cells. The data mean ± SD is representative of six sets of independent experiments for healthy women and CaBr patients separately

patients as compared with that induced by MIP1α alone, MIP1β alone, or IFN-γ alone. IL-4 alone inhibited cytotoxicity of the PBM of both healthy women and CaBr patients. In the presence of IL-4, MIP1α and MIP1β failed to induce cytotoxicity in the PBM of both healthy women and CaBr patients (Fig. 6b). The synergy of IFN-γ with MIP1α and MIP1β and downregulation by IL-4 was also found in the induction of O_2^- and TNF release by the PBM of healthy women and CaBr patient's data. In all the assays, the synergy of IFN-γ was found to be greater with MIP1α than with MIP1β.

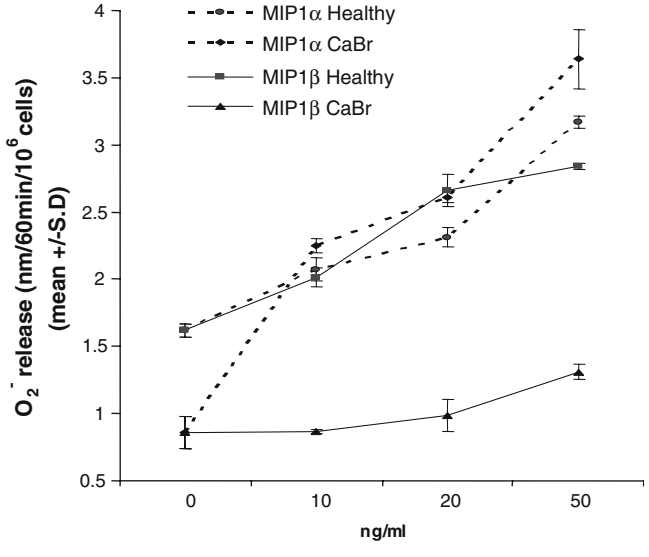

Fig. 4 MIP1α and MIP1β differentially modulate superoxide anion release from the PBL of healthy women and breast cancer patients. The PBM of six healthy women and six CaBr patients were cultured for 18-h in the presence or absence of 10–50-ng/ml MIP1α or MIP1β. The O_2^- released in the culture supernatants was measured by SOD inhibitable Ferricytochrome C reduction. The data represents mean + SD of six sets of independent experiments for healthy women and CaBr patients separately

Discussion

The pro-inflammatory C–C chemokines MIP1α and MIP1β are potent chemoattractants for the mononuclear cells [32]. The MIP1β, when injected causes a mild neutrophil accumulation followed by a more prominent monocytic infiltration [3, 27], a process that involves the activation of the cells. Appropriately, the activated monocytes and tissue macrophages release cytokines and have the ability to kill tumor cells [10, 45]. The monocytes, which are a major source of MIP1α and MIP1β, thus may play an important role in malignancy-associated inflammatory and immune responses, and the control of tumor growth.

Initial insult results in the release of proinflammatory cytokines, IL-1 and TNF, from the endothelial cells and tissue macrophages, which in turn induce the release of C–C and C–X–C chemokines from the surrounding stromal or parenchyma cells. The production of IL-1 was shown to be a necessary intermediate step for MCP-1 gene expression in the endothelial cells [37]. The C–C

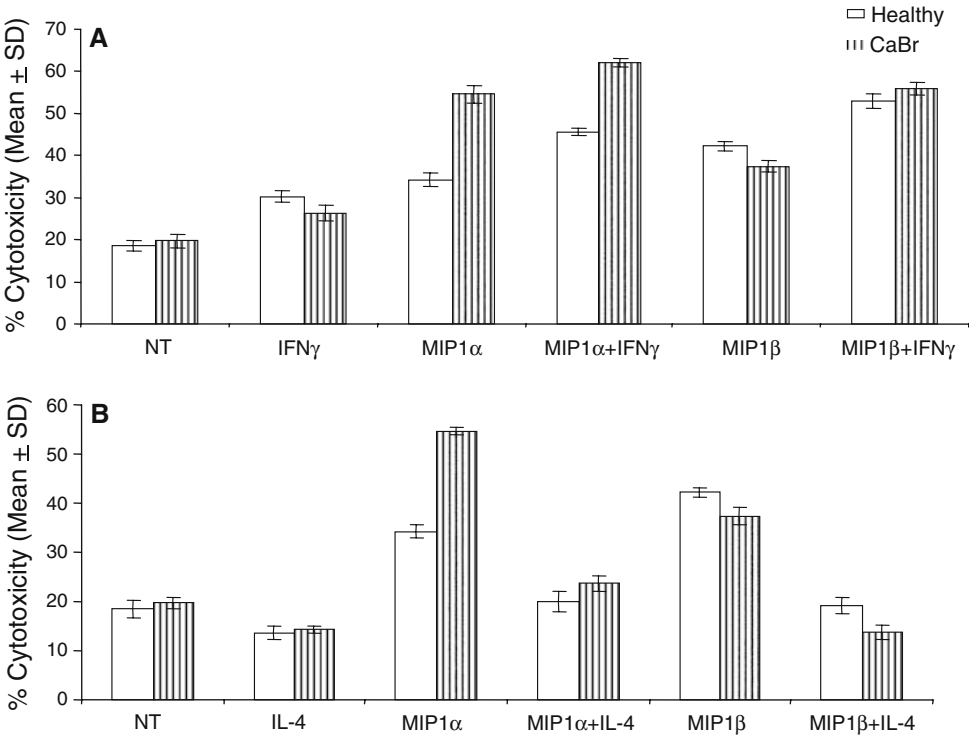

Fig. 6 IFN-γ upregulates and IL-4 downregulates MIP1α- and MIP1β-induced activation of PBM. **a** IFN-γ synergizes with MIP1α and MIP1β in inducing cytotoxicity in PBM. **b** IL-4 downregulates MIP1α- and MIP1β-induced cytotoxicity of PBM. The PBM of six healthy women and six CaBr patients were cultured with MIP1α (50 ng/ml) alone, MIP1β (50 ng/ml) alone, IFN-γ (200 U/ml)) alone, IL-4 (200 U/ml) alone, or with the combined doses of the IFN-γ or IL-4 with MIP1α and MIP1β for 18 h. The PBM were then co-cultured with T24 cells at an E:T of 20:1 for 4 h and LDH released specifically from the target cells was measured by using LDH assay kit to determine cytotoxicity of the PBM. The data (mean ± SD) is representative of six sets of independent experiments in healthy women and CaBr patients separately

chemokines activate and recruit PBM and lymphocytes, which further release inflammatory cytokines and chemokines, and induce inflammatory and immune responses. The IL-1 and TNF released by the activated monocytes also regulate adherence to endothelium and transendothelial migration of leukocytes by enhancing the expression of adhesion molecules in both leukocytes and endothelial cells [17, 21, 34]. Though MCP-1 regulates the induction of adhesion molecules it is unable to induce TNF release [22]. Fahey et al. [16] using mouse peritoneal exudate cells observed that MIP1α, but not MIP1β, induced the release of IL-1 and TNFα. In contrast, the present data show that IL-1 and TNF release from the PBM of healthy women was markedly enhanced by MIP1β and only marginally by MIP1α (Figs. 1, 3). On the other hand, MIP1α had a marked stimulatory effect on the release of these cytokines by the PBM of CaBr patients, but MIP1β had no effect on these cells (Figs. 1, 3). IL-1 secretion by the PBM in response to LPS also differed in healthy women and CaBr patients (Fig. 2). LPS, like IL-1, activates a protein kinase cascade that leads to altered gene expression. P38, an MAP kinase family member found in the cytoplasm and the nucleus of activated cells, is phosphorylated in tyrosine residues in response to LPS [19]. The blocking of the MAP kinase by a pyridinyl amidazol compound inhibited IL-1 and TNF release by LPS-stimulated monocytes [19, 23]. The cell surface changes and the deficient kinase activation associated with malignancy [24] may be responsible for the observed deficiency in LPS, as well as MIP1β, and also the induced IL-1 secretion by the PBM of the CaBr patients. Similar to the synergistic effect of MCP-1 with LPS in the induction of tumoricidal macrophages [20], MIP1α showed synergy with LPS in inducing IL-1 from the PBM of both healthy women and CaBr patients (Fig. 2). The receptor for MIP1α, CCR1, couples to multiple G proteins Gi and Gq. The Gi coupled receptors are known to stimulate MAPK via $\beta\gamma$ subunits [14]. The synergy between MIP1α and LPS in stimulating the IL-1 release may be due to the activation of common MAPK family members by CCR1 and LPS. Although CCR5, the receptor for MIP1β, signals through Gγ i proteins and phosphorylates the MAPK family [4, 18], no synergy between MIP1β and LPS was observed.

While some chemokines may favor tumor growth and metastasis by promoting angiogenesis and tumor-cell proliferation, others may enhance innate or acquired host immunity against tumor [43]. The MIP1α, MIP1β, and RANTES are known to augment NK cell cytotoxicity [41]. Like other agonists [46], MIP1α and MIP1β also induced respiratory bursts in the monocytes (Fig. 3). The augmented release of TNFα and O_2^- by the PBM of healthy women (Figs. 3, 4) correlated with their enhanced tumor killing ability induced by MIP1α and MIP1β (Fig. 5). The specificity of MIP1α- and β-induced cytotoxicity of the monocytes was evident in the blocking of the chemokine-induced tumor target killing by the monocytes with MIP1α and MIP1β antibodies. It appears that the PBM-mediated killing of T24 cells involved TNFα-independent mechanisms, as in the CaBr patients, the PBM were found to be deficient in secreting TNFα (Fig. 3) and O_2^- (Fig. 4), but not in killing T24 cells (Fig. 5).

The activation of monocytes to classical inflammatory macrophages is regulated by the TH-1 cytokines.

The TH-1 effector cells are recruited in the inflamed sites by the C–C chemokines [2], which are inducible and upregulated in inflammatory lesions. The MIP1α is highly effective in recruiting TH-1 cells [39] and it preferentially chemo attracts $CD8^+$ cells [40]. The influx of $CD8^+$ cells, a major source of MIP1α [11], into the tumor is associated with the restriction of tumor growth [15]. The synergy observed between IFN-γ and MIP1α or MIP1β in augmenting tumor killing (Fig. 6a) and the release of TNFα and O_2^- (data not shown) by the PBM of both healthy women and CaBr patients suggests that the C–C chemokines and the TH-1 cytokines may regulate monocyte activation through their concerted effort. The present data showed more marked synergy of IFN-γ with MIP1α than with MIP1β, the underlying mechanism of which is not clearly understood. The G-protein coupled C—C-chemokine receptors activate phospholipase C, inositol tryphosphate, intracellular Ca^{2+} mobilization, and protein kinese C (PKC) [7]. The activation of common signaling molecules, such as PKC, by the C–C chemokines and IFN-γ may account for their co-stimulatory effect in inducing tumoricidal monocytes. The synergy of IFN-γ with MIP1α and MIP1β in activating the PBM (Fig. 6a) may also be due to IFN-γ-induced increased expression of CCR1, the receptor for MIP1α, and CCR5, the receptor for MIP1β and MIP1α in these cells [12, 35, 47].

The human leukocytes were shown to produce C–C chemokines during type I response but not during type II response [3]. We have earlier reported downregulation of IL-12 and IFN-γ release by the PBM and PBL in CaBr patients [7], which may be correlated with the malignancy-associated preponderance of TH-2 responses [5], and may explain the observed downregulation of monocyte activation in CaBr patients. The suppression of MIP1α- and MIP1β-induced PBM cytotoxicity by IL-4 (Fig. 6b) suggests that the TH-2 cytokines, similar to their inhibitory effect on the TH-1 cytokine-induced monocyte activation [1, 30], may downregulate the C–C chemokine-induced activation and generation of tumoricidal monocytes.

The role of the communicating network of C–C chemokines and TH-1 cytokines as a regulator of cell-mediated immune responses and inflammatory responses in cancer is also evident from the findings that the CCR1 and CCR5 are expressed in both monocytes and TH-1 cells [35, 36].

Our results show that MIP1β is more potent in activating the PBM of healthy women than MIP1α, whereas in breast cancer patients, MIP1α is more effective than MIP1β (Figs. 1–5). Using RNA–DNA hybridization and RT-PCR techniques, we have observed overexpression of CCR1 and downregulation of CCR5 in the PBM of CaBr patients as compared with those in the PBM of healthy women, which correlated well with the observed differential effect of MIP1α and MIP1β on these cells. The RT-PCR analysis also showed the same results as of dot blot DNA–RNA hybridization. The present findings provide evidence that MIP1α, along with TH-1 cytokines, plays a greater role than MIP1β in monocyte- mediated regulation of tumor growth.

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