Effect of cigarette smoke on eosinophil function *in vitro*

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Increased eosinophil counts have been described in active and passive smokers. Eosinophil inflammation has been found in the bronchial mucosa of smokers with chronic bronchitis. In an animal model, repeated exposure to cigarette smoke increased bronchial hyperreactivity together with increased eosinophils and macrophages in the BAL. We therefore investigated the effect of cigarette smoke extract (CSE) *in vitro* on eosinophil function.

In three experiments we studied eosinophil (EOS) survival in the presence of CSE at 1% and 0.1% concentrations, corresponding to 10^{-6} and 10^{-7} M nicotine/ml. Spontaneous survival at 72 h was $60 \pm 23.3\%$. EOS survival was increased by IL-5 0.1 ng/ml ($72.2 \pm 10.3\%$) and by GM–CSF 1 pM/ml ($71.5 \pm 6\%$). CSE (1%) did not influence EOS survival at 24 or 48 h. At 72 h, EOS survival was significantly decreased by CSE (1%) ($27.0 \pm 20.3\%$, $p < 0.01$). However, both IL-5 ($61.1 \pm 13\%$) and GM–CSF ($75 \pm 4.8\%$) significantly suppressed the toxic effect of CSE (1%) ($p < 0.03$). CSE (0.1%) did not decrease EOS survival at 72 h. We investigated EOS adhesion to respiratory epithelial cells (REC). REC (A549 immortalized cell line) were grown to confluence in 96-well cell culture plates, and then treated for 24 h with CSE (0.1) (nicotine 10^{-5} M), TNFα (250 U/ml), or the combination of TNFα and CSE at the same concentrations. The expression of ICAM-1 was measured by whole-cell ELISA. As described previously, TNFα significantly increased the expression of ICAM-1 (0.54 ± 0.06, $p < 0.0001$). However, CSE (0.77 ± 0.07) further increased the TNFα-induced expression of ICAM-1 ($p < 0.0004$). EOS adhesion to A549 was investigated under the same conditions, and measured using an eosinophil peroxidase (EPO) based assay. In four experiments, EOS adhesion to TNFα treated A549 was increased by CSE (13 ± 5.2 *vs.* $14.9 \pm 4.8\%$, $p < 0.01$. In two experiments, the increased EOS adhesion induced by TNFα and CSE was inhibited by anti-CD18 (7.1 ± 2.7 *vs.* $13 \pm 5.2\%$ and 8.03 ± 2.9 *vs.* $14.9 \pm 4.8\%$) suggesting the Mac-1-ICAM-1 interaction takes part in this process. We conclude that in these experiments the toxic effect of CSE (1%) on EOS survival is suppressed by IL-5 and GM-CSF. CSE enhances the expression of ICAM-1 induced by TNFα, and increases eosinophil adhesion to TNFα treated cells. These findings are possibly relevant to the role of eosinophil inflammation in passive and active smokers.

Sputum eosinophilia and bronchial hyperresponsiveness (BHR) in asthma

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We compared induced sputum cytology in stable asthmatics (ten allergic and eight non-allergic) and in eleven normal subjects. Sputum was obtained after inhalation of hypertonic saline solution (5%). Cell counts and differentials were determined. Each subject underwent pulmonary function tests and methacholine responsiveness was measured using the method of Cockroft. We found that sputum induction with NaCl (5%) is a non-invasive alternative for collecting bronchial lining fluid in asthmatic subjects. As previously described by others, we found a higher percentage of eosinophils in sputum from asthmatics than from normal subjects ($25 \pm 3.2\%$ *vs.* $1.6 \pm 0.4\%$, $p < 0.05$). The inflammatory response (% eosinophils and neutrophils) tends to be stronger in non-allergic than in allergic asthmatics. The percentage of eosinophils was inversely correlated to the degree of airway obstruction (VEMS/CV) ($r = -0.63$, $p < 0.05$). No correlation was found between sputum eosinophilia and BHR. These observations suggest a more severe bronchial inflammation in non-allergic asthma and support the view that other factors unrelated to the number of sputum eosinophils may contribute to the onset and maintenance of bronchial hyperresponsiveness.

Cytokines and cartilage degradation

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Human articular chondrocytes isolated from their matrix by three successive enzymatic digestions were cultured for 24 to 72 h, under constant agitation in DMEM supplemented with 1% Ultroser G.

Recombinant IL-1β (1, 10, 50 ng/ml), TNFα (2, 20, 100 ng/ml), IL-6 (2, 20, 200 ng/ml), IL-8 (0.8, 8, 80 ng/ml) and LIF (1, 10, 50 ng/ml) were investigated for their actions on proteoglycan (PG), prostaglandin E2 (PGE_2) and cytokine production. At each concentration used in this study, IL-1β decreased PG production and increased PGE_2, IL-6, IL-8 and LIF. TNFα had similar effects to IL-1β on cytokine and PG production. In these culture conditions, TNFα has no effect on PGE_2 production. Interestingly, IL-6 decreased IL-8 production by chondrocytes. IL-8 is an important chemoattractant agent for neutrophils and T-lymphocytes. In this way, IL-6 could limit chondroresorption. IL-8 induced a moderate increase of IL-6 production and had no effect on LIF production. On the other

hand, IL-6 and IL-8 did not modify PG and PGE_2 production. Finally, we observed a stimulation of IL-1β production by LIF (20 ng/ml). LIF also increased IL-6 and IL-8 production and partially inhibited PG synthesis. Meanwhile, using polyclonal antibodies neutralizing IL-1β and α activities, we demonstrated that IL-1 mediates LIF effects.

In conclusion, IL-1β and TNFα are potent mediators of cartilage degradation, not only by their deleterious effects on matrix synthesis but also by the over-production of IL-6, IL-8 and LIF. These cytokines may act by autocrine, paracrine and systemic regulation on the development of arthropathies.

Ex vivo cytokine production by whole blood cells from cancer patients

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It has been hypothesized that the development of cancer could partially result from a diminution of immunocompetence. Using *ex vivo* cytokine production by whole blood (WB) cells after polyclonal activation, we compared cytokine production levels of cancer patients with those of healthy controls. Seventeen patients without any prior treatment and attending the hospital for oncological surgery (for cancers of several origins) were enrolled in the study. WB was collected in heparinized tubes, diluted 1/10 in RPMI 1640 and incubated for 2, 4, 24, 48 and 72 h at 37°C in the presence of 5 μg/ml PHA and 25 μg/ml LPS. Cytokine levels in the supernatant were measured by specific immunoassay kits. IL-10 levels after 24 h of culture, IFN-γ and GM–CSF levels after 24 and 72 h of culture, and LIF levels after 72 h of culture were significantly lower in cancer patients compared to healthy controls. No significant difference was observed for IL-1β, IL-2, IL-4, IL-6, IL-8 and TNFα production at any culture time. Our results suggest that the putative immunosuppression of cancer patients might be reflected by their reduced production of immunostimulated cytokines.

Modulation of human cultured fibroblasts cell redox potential: effects on the transcriptional factor NFkappaB activated by IL-1

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Reactive oxygen intermediates (ROI) have been shown to be involved in the activation of NFkappaB, a potent and pleitropic transcription factor, activated among others by interleukin-1 (IL-1). As it has been mainly studied in immune system-derived cells, we wanted to investigate the activation mechanisms of NFkappaB when stimulated by IL-1 in human fibroblasts.

NFkappaB is indeed activated by IL-1 in these cells, and we then tested whether any modulation of the cells' main antioxidant enzymes would affect the NFkappaB activation and cell response induced by IL-1. In the first approach, the effects of different specific inhibitors (that is, mercaptosuccinate for glutathione peroxidase, aminotriazole for catalase, and bischlorethylnitrosourea for glutathione reductase) were tested on the activation of NFkappaB induced by IL-1. In the second approach, a transfected cell line of SV40 transformed fibroblasts over-expressing glutathione peroxidase was used and the behaviour of these cells regarding IL-1-induced NFkappaB activation was compared to the corresponding non-transfected cells.

Cytokine modulation of gelatinase A activation in the fibrosarcoma HT1080 cell line

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Gelatinase A (Mr 72 000 type IV collagenase) is a member of the matrix metalloproteinase family, which is synthesized and secreted as a latent proenzyme. The removal of an 80 amino acid *N*-terminal domain yields a Mr 62 000 active enzyme capable of participating in extracellular matrix remodelling, which occurs in normal and pathological processes. The activation of gelatinase A *in vitro* is induced by treatment with the organomercurial compound *p*-APMA. Little is known about the physiological mechanism of the activation process and its regulation. In contrast to the other matrix metalloproteinases, gelatinase A activation does not involve proteolytic cleavage by stromelysin or serin proteases. It requires prior binding of the proenzyme to the cell plasma membrane where it is processed to its lower molecular mass activated form. In the present study, we investigated the influence of the cytokines epidermal growth factor (EGF), tumour necrosis factor (TNFα) and interferon (INF-γ) on the secretion of gelatinase A by the invasive HT1080 fibrosarcoma cell line and on the cell membranes activation capacities. The obtained results indicate that EGF, TNFα and INF-γ induce secretion of activated gelatinase A by the HT1080 cell line. The three cytokines show differential effects on the membrane activation of gelatinase A.