β-GLUCANS MODULATE ALVEOLAR MACROPHAGE RESPONSE TO BACTERIAL LIPOLYSACCHARIDE. QA Hoffman, JE Standing, AH Limper. Thoracic Disease Research Unit, Mayo Clinic and Foundation, Rochester MN.

Tumor necrosis factor-alpha (TNFα) is a potent proinflammatory cytokine believed to play a central role in the pathogenesis of endotoxin-induced shock and the adult respiratory distress syndrome. Pretreatment of animals with β-glucan prior to bacterial challenge reduces detectable TNFα and lethal infection (J. Cell. Biochem., 1991; 46: 60). We therefore hypothesized that β-glucan might directly regulate TNFα secretion from alveolar macrophages (AMS) in response to lipopolysaccharide (LPS). Rat AMS were cultured in the presence of increasing concentrations of β-glucan alone and TNFα secretion quantified using a sensitive L929 cytotoxicity assay. Whereas unstimulated AMS released 22±2.3 pg/ml of TNFα, addition of β-glucan at doses of 10 and 100 μg/ml resulted in suppression of TNFα release from AMS (15.0±1.6 pg/ml; p<NS compared to controls). Furthermore, preincubation of AMS with 500 μg/ml of β-glucan also inhibited the secretion of TNFα induced by LPS (10 μg/ml). Whereas LPS-stimulated AMS released 13,407±248 pg/ml of TNFα, AMS pretreated with β-glucan released only 4.0±0.2 pg/ml. Additionally, interferon-γ (INF) (10ng/ml), a potent activator of TNFα expression, failed to overcome the inhibition of TNFα release induced by β-glucan. Our study demonstrates that β-glucan regulates the secretion of TNFα from AMS. Lower concentrations of β-glucan itself stimulate TNFα release, however higher concentrations of β-glucan inhibit TNFα secretion. Furthermore, AMS suppressed by β-glucan are also refractory to further stimulation by LPS even following IFNγ priming. These data suggest an immunomodulatory role of β-glucan which may explain its beneficial effect in models of sepsis.

POTENTIAL, INDIRECT ANTI-INFLAMMATORY EFFECTS OF IL-4: STIMULATION OF HUMAN MONOCYTES, MACROPHAGES, AND ENDOTHELIAL CELLS BY IL-4 INCREASES AMINOPEPTIDASE-N-ACTIVITY (CD13; EC 3.4.11.2)

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IL-4 up-regulates various monocyteic properties, which are associated with pro-inflammatory functions. Paradoxically, IL-4 may also act as an anti-inflammatory agent by down-regulating the production of several inflammatory mediators. Studies have already been started to examine these properties in vivo. As the activity of some mediators has recently been shown to be regulated by peptidases, we examined whether IL-4 was able to modulate the expression of a cell membrane associated peptidase, aminopeptidase-N (CD13).

Monocytes were isolated from healthy volunteers, purified by centrifugal elutriation, and cultured under non-adherent conditions in Teflon bags. Expression of cell surface antigens was analyzed with a FACScan. IL-4 increased aminopeptidase activity of monocytes, aminopeptidase-N (CD13), measured by the appearance of autoradiography. The apparent expression of aminopeptidase-N on the monocyte cell surface was increased by the addition of IL-4. The marked increase in aminopeptidase activity of monocytes was induced by IL-4. Also other CD13-expressing cells were sensitive to IL-4, since CD13 Ag expression and CD13 mRNA levels were up-regulated in human alveolar macrophages and endothelial cells upon IL-4 treatment.

The increased expression of cell membrane aminopeptidase-N represents a potentially increased cellular ability to inactivate inflammatory mediators. Therefore, these findings may be of potential importance for the understanding of antinflammatory actions. We postulate that up-regulation of aminopeptidase-N expression may be an indirect working mechanism of IL-4 to modulate the action of bioactive peptides. This mechanism as such may also be relevant in studies on the anti-inflammatory effects of IL-4 in vivo.

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TGF-β and TNF-α INFLUENCE FIBRIN TURNOVER IN HUMAN TRACHEAL EPITHELIAL CELLS IN VITRO. Johnson, AR, Koenig, KB, and Idell, S. University of Texas Health Cnr,Tyler, TX, USA.

Alveolar fibrin deposition characterizes alveolitis of severe forms of lung injury and may potentiate inflammation and subsequent alveolar organization. The epithelial lining of small airways and alveoli can influence local fibrin deposition via expression of procoagulant and fibrinolytic proteins. We used a human tracheal epithelial cell line (HEPC) to study cytokine influence on fibrin deposition. From recalcification times in factor-deficient plasmas we found that tissue factor (TF) accounts for most procoagulant activity of HEPC and confirmed this by direct and indirect binding studies with factor VII and neutralization with TF antibody. TGF-β and TNF-α stimulated release of TF into HEPC-conditioned media. HEPC expressed plasminogen-dependent fibrinolytic activity which, by fibrin enzymography, was primarily uPA; some remained in complex with inhibitor(s). TGF-β and TNF-α altered expression of fibrinolytic proteins and inhibitors in HEPC as measured by fibrin radio-assay and ELISAs. TGF-β depressed fibrinolytic activity in both cells and media. By contrast, TNF-α increased both cell-associated and media fibrinolytic activity. TNF-α also induced increased expression of uPA and tPA in HEPC, whereas TGF-β did not. TGF-β enhanced both cell-associated and media PAI-1. PAI-1 induction may be relevant in studies on the anti-inflammatory effects of TGF-β and TNF-α differences from other human lung cells (mesothelium and fibroblasts) we studied previously. Although induction of procoagulant activity and depression of fibrinolysis by TGF-β favors fibrin deposition, the induction of fibrinolytic activity by TNF-α could shift the balance towards fibrin clearance.

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MODULATION BY IL-10, IL-4 and TGFβ OF TNFα, TGFβ AND T CELL PROLIFERATION INDUCED BY ALLOGENEIC ALVEOLAR MACROPHAGES OR DENDRITIC CELLS.

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TNFα and TGFβ are powerful inflammatory and cytotoxic cytokines that may play a key role in acute or chronic lung rejection. We have studied their release during allogenic reactions induced by either human lung dendritic cells (DC) or alveolar macrophages (AM). The modulation of their production by IL-10, IL-4 or TGFβ was analyzed. DC were separated using their density, their adherence to plastic and their absence of autologous monocyteic inclusions. AM or DC (2x10^5) obtained from the same surgical specimens were mixed with allogenic T Cells (150x10^3). Supernatants were collected during five consecutive days. The maximal concentration of TNFα and β was measured on the fifth day by RIA (Medgenix) and ELISA (R and D), respectively. TNFα levels were 169±133 pg/ml (+ SEM) and 513±64 pg/ml when T cells were mixed with DC and AM (n = 6), respectively. The TNFα levels were 786±187 pg/ml with DC-T Cells and 100±57 with AM-T Cells. When IL-10, IL-4 or TGFβ was added to the alloreactions only IL-10 (10 ng/ml) reduced T cell proliferation (expressed in cpm) as well as TNFα or β as shown here in a representative experiment:

<table>
<thead>
<tr>
<th>DC + T</th>
<th>DC + T + IL-10</th>
<th>AM + T</th>
<th>AM + T + IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>143±28</td>
<td>25±50</td>
<td>13±5</td>
<td>3±75</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>2.85±4</td>
<td>60 ± 500</td>
<td>278</td>
</tr>
<tr>
<td>TGFβ (pg/ml)</td>
<td>34±9</td>
<td>56 &lt;15</td>
<td>&lt;15</td>
</tr>
</tbody>
</table>

With IL-4 T cell proliferation as well as TNFα production were enhanced by 60 ±15% and 275 ± 22% respectively in the presence of DC whereas TNFα concentrations were not significantly changed. With TGFβ T cell proliferation and TNFα production were slightly decreased by 30 ±11% and 28 ± 35% respectively, whereas TNFα was increased by 95 ± 27%. Thus IL-10, contrarily to IL-4 or TGFβ production decreased T cell proliferation as well as TNFα or TGFβ during allogenic reaction.

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