The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/27165

Please be advised that this information was generated on 2020-01-11 and may be subject to change.
β-GLUCANS MODULATE ALVEOLAR MACROPHAGE RESPONSE TO BACTERIAL LIPOPOLYSACCHARIDE. 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 (AM) in response to lipopolysaccharide (LPS). Rat AM were cultured in the presence of increasing concentrations of β-glucan alone and TNFα secretion quantified using a sensitive L929 cytotoxicity assay. Whereas unstimulated AM released 22.7 ± 5.3 pg/ml of TNFα, addition of β-glucan at doses of 10 and 100 μg/ml resulted in increased TNFα release (131.1 ± 26.1 and 729.9 ± 294.3 pg/ml TNFα respectively; p < 0.05 compared to controls). However, lower concentrations of β-glucan (<500 μg/ml) resulted in suppression of TNFα release from AM (15.0 ± 1.6 pg/ml; p = NS compared to controls). Furthermore, preincubation of AM with 500 μg/ml of β-glucan also inhibited the secretion of TNFα induced by LPS (10 μg/ml). Whereas LPS-stimulated AM released 13,407 ± 248 pg/ml of TNFα, AM pretreated with β-glucan released only 4.0 ± 0.2 pg/ml. Additionally, interferon-gamma (INF) (10 μg/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 AM. Lower concentrations β-glucan itself stimulate TNFα release, however higher concentrations of β-glucan inhibit TNFα secretion. Furthermore, AM suppressed by β-glucan are also refractory to further stimulation by LPS even after IFN 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).

Depts. of Immunology1 and Pulmonary Medicine2, Erasmus University and University Hospital Dijkzigt, Rotterdam; Dept. of Immunology3, Antoni van Leeuwenhoek Huis, Amsterdam, The Netherlands.

IL-4 up-regulates various monocyteic properties, which are associated with proinflammatory 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 elutiation, and cultured under non-adherent conditions in Teflon bags. Expression of cell surface antigens was analysed with a FACScan. IL-4 caused a dose-dependent increase of the expression of CD13 Ag on these cells. Maximal expression was observed around 48 h of culture. This IL-4-induced increase was completely blocked by anti-IL-4 antisera. Furthermore, the increase in surface expression was preceded by increased mRNA levels of CD13, which was maximal around 24 hours of culture. We also observed that CD13-mediated leucine-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 provide further insights into the role of IL-4-mediated anti-inflammatory 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.

Supported by Glaxo Holland b.v.

ENDOTHELIAL CELLS BY IL-4 INCREASES AMINOPEPTIDASE-N-ACTIVITY (CD13; EC 3.4.11.2).

TCGF-β and TNF-α INFLUENCE FIBRIN TURNOVER IN HUMAN TRACHEAL EPITHELIAL CELLS IN VITRO. Johnson, AR, Koenig, KB, and Iedell, S. University of Texas Health Ctr, 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 studied a human tracheal epithelial cell line (HEPC) to study cytokine influence on fibrin deposition. From re-calification times in factor-deficient plasma 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. IL-4β 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). TCGF-β and TNF-α altered expression of fibrinolytic proteins and inhibitors in HEPC as measured by fibrin radio-assay and ELISAs. TCGF-β depressed fibrinolytic activity in both cells and media. By contrast, TNF-α increased both cell-associated and media fibrinolytic activity.

IL-10 also induced increased expression of uPA and tPA in HPEC, whereas TCGF-β did not. TCGF-β enhanced both cell-associated and media PAI-1. PAI-1 induction was not dependent on cytokine expression, since PAI-1 induction was similar in cells expressing high or low cytokine. TCGF-β also down-regulated uPA expression. TCGF-β and TNF-α decreased PAI-1 expression, which may explain its beneficial effect in models of sepsis.

Supported by NIH grants HL45018, HL37770 and HL44473.

MODULATION BY IL-10, IL-4 AND TGF-β OF TNFA, TGF-β AND T CELL PROLIFERATION INDUCED BY ALLOGENIC ALVEOLAR MACROPHAGES OR DENDRITIC CELLS.

L.F. Wierda1, F. El Habre, J.-M. Dever Division of Respiratory Diseases1 and Division of Immunology and Allergy, University Hospital, Geneva, Switzerland.

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 size and the abundance of autofluorescent phagocytic inclusions. AM or DC (20x103) obtained from the same surgical specimens were mixed with allogenic T Cells (15x103). Supernatants were collected during five consecutive days. The maximal concentration of TNFα and β was measured the fifth day by RIA (Medgenix) and ELISA (R and D, respectively). TNFα levels were 1694 ± 133 pg/ml (+ SEM) and 513 ± 64 pg/ml when DC or AM were mixed with DC and AM (+), 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 (10ng/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>CPM</th>
<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>DC</td>
<td>1000</td>
<td>2200</td>
<td>1500</td>
<td>1200</td>
</tr>
<tr>
<td>TNFα</td>
<td>43.2%</td>
<td>25.502</td>
<td>13.151</td>
<td>3.793</td>
</tr>
<tr>
<td>TNFβ</td>
<td>2.854</td>
<td>60</td>
<td>500</td>
<td>278</td>
</tr>
<tr>
<td>TNFβ</td>
<td>3.34</td>
<td>56</td>
<td>&lt;5</td>
<td>&lt;15</td>
</tr>
</tbody>
</table>

With IL-4 T cell proliferation as well as TNFα production were enhanced by 60 ± 16% 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 TNFβ during alloreactive reaction.

Supported by the FNRS N° 313019.91 (IN) and 31-33786.92 (IN)