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SYSTEMIC AND CELLULAR RELEASE OF IL-10 IN HUMAN BMT

E Holler, MG Roncarolo, R Hintermeier-Knabe, B Ertl, E Rustige, M Albiez, HJ Kolb, W Wilmanns (Supported by DFG-grant Ho 1142)

In previous studies, our group has demonstrated involvement of early release of TNFalpha in induction of GvHD and severe transplant related complications (TRC) in pts receiving allogeneic BMT. As IL-10 is a major endogenous TNF-antagonist, we now prospectively monitored systemic (n=40 pts) as well as cellular (n=76 pts) release of IL-10 starting from admission until d 30 following BMT using a sensitive ELISA. During conditioning and in the first month following BMT IL-10 serum levels significantly increased in pts developping TRC such as severe GvHD, sepis or renal failure. After engraftment, spontaneous production of IL-10 by PBMNC was increased in pts with clinical GvHD and suppressed following treatment with high dose corticosteroids. These data clearly indicate reactive release of IL-10 in the course of inflammatory conditions. In contrast, cellular production of IL-10 in clinically asymptomatic pts at the time of admission was signifcantly elevated in pts with subsequent unveventful courses ($p < 0.005$) as compared to pts with later TRC. Increased IL-10 production in pts with uneventful courses could not be explained by clinical factors but showed a close correlation with spontaneous cellular production of TNFalpha and HLA-DR subtypes. These data suggest immunogenetically defined subgroups of pts characterized by high spontaneous TNF and IL-10 production which are protected from further cytokine mediated damage in the course of BMT. Due to a low incidence of GvHD, IL-10 might even mediate tolerance in this particular subgroup of pts.

Med Klinik III, Klinikum Großhadern und Inst f Klinische Hämatologie der GSF, 81377 München; DNAX Research Inst., Palo Alto, California

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AN AUTOCRINE FACTOR OF AN OVARIAN CARCINOMA CELL LINE INDUCES C-MYC TRANSCRIPTS, DNA-SYNTHESIS AND APOPTOSIS.

G. Krupitza[1], H. Harant[1], E. Dittrich[2], H. Huber[2], T. Szekeres[3] and C. Dittrich[1].

A homogenous tumor cell line - HOC-7-N.1, derived from a human epithelial ovarian adenocarcinoma, which is fast growing and which represents an undifferentiated phenotype secretes a factor which autocrinely stimulates the growth associated oncogene c-myc. A sister cell line, which was derived from the same tumor grows slowly, exhibits a differentiated morphology and does not produce a c-myc stimulatory factor. It was found that the activity of the autocrine factor was inhibited by antibodies specific against macrophage-colony stimulating factor (CSF-1). There is evidence that CSF-1 and its receptor, the c-fms oncogene are indeed expressed in HOC-7-N.1 cells. The factor accumulates in the culture medium when fetal calf serum (FCS) is omitted just as in FCS containing medium. Preliminary studies show that the presence of FCS is not required to induce c-myc m-RNA by the secreted factor. Upon stimulation with the autocrine factor HOC-7-N.1 cells undergo programmed cell death under serum-free conditions, however.
We think that induction of apoptosis in HOC-7-N.1 cells is the consequence of increased c-myc expression in absence of survival factors.

[1],Ludwig Boltzmann Inst. of Applied Cancer Research, 3rd Med. Dept. with Oncology, Kaiser Franz Josef-Hospital, Vienna,
[2],Dept. Int. Med.I, Div. Oncol, Univ. Vienna,
[3],Inst. of Medical Chemistry, Univ. Vienna;
Vienna, Austria.

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BACTERIAL ENDOTOXIN SENSITIZES HUMAN ENDOTHELIAL CELLS AGAINST RADIATION-INDUCED PROGRAMMED CELL DEATH THROUGH THE ACTION OF TRANSMEMBRANE TNF-α

G. Eißner*, F. Kohlhuber#, M. Grell+, P. Scheurich+, A. Hieke*, G.W. Bornkamm*, E.Holler**

In this study we show that bacterial endotoxin (LPS) was able to enhance apoptosis in human endothelial cells mediated by ionizing radiation (IR) in a clinically relevant dose (4Gy). In contrast, LPS alone was unable to induce programmed cell death. Coincubation of LPS+IR-treated endothelial cells with the antagonistic cytokine Interleukin 10 (IL-10) revealed the involvement of proinflammatory cytokines in this process, since IL-10 abrogated the LPS-triggered enhancement of IR-induced apoptosis. Further analysis showed that tumor necrosis factor α (TNF) was responsible for the sensitizing effects mediated by LPS, but not for the IR-mediated programmed cell death. Furthermore, it turned out that the transmembrane form of TNF, not soluble TNF (sTNF), contributed to apoptotic cell signalling, since exogenous sTNF, even in high concentrations, proved to be ineffective in this system. Finally, TNF-receptor TR60 could be identified as the relevant target structure for mTNF, whereas TR80 did not appear to be involved in endothelial cell apoptosis.
These findings seem of clinical importance as LPS+IR-induced apoptosis might contribute to the endothelial damage following radiotherapy or total body irradiation, especially in association with endotoxemia or other inflammatory disorders.

GSF-Institut für Klinische Molekularbiologie* und Hämatologie, Marchioninistr. 25, D-81377 München, Germany; Biochemical Regulatory Mechanisms Laboratory#, ICRF, London, England; Institut für Immunologie und Zellbiologie+, Universität Stuttgart, Germany.**

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RECEPTOR BINDING, ACTIVATION OF cAMP DEPENDENT PROTEIN KINASE (PKA) SIGNAL TRANSDUCTION PATHWAYS AND INDUCTION OF INTERLEUKIN-8 (IL-8) mRNA BY A (1→3)-β-D-GLUCAN BIOLOGICAL RESPONSE MODIFIER IN THE HUMAN MONOCYTE-LIKE CELL LINE U937. A. Müller, C. Portera, E. Love, H. Ensley, J. Kelley, T. Ha, P. Rice, R. Orcutt, W. Browder and D. Williams. Dept. of Surgery, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614-0575, and Dept. of Chemistry, Tulane University, New Orleans, LA, 70115, USA.

Carbohydrate polymer based biological response modifiers (BRMs) have been demonstrated to exhibit immune stimulatory activity. Prophylaxis or therapy with (1→3)-β-D-glucan BRMs has been shown to be beneficial in the treatment of wounds, surgical sepsis and neoplasia. Glucans induce immune stimulation, in part, via macrophage activation. The cellular and molecular mechanisms of glucan induced macrophage activation have not been elucidated. This study examined the specificity of receptor-binding, activation of signal transduction pathways and induction of cytokine mRNA by glucan in cultured U937 cells. We prepared labeled [^{3}H]-glucan phosphate with a M_w of 1.86 x 10^5 g/mol, polydispersity of 2.03, radius of gyration of 24.7 nm and intrinsic viscosity of 37.1 ml/g. Cells (1 x 10^6/well) were co-incubated with [^{3}H]-glucan phosphate (300 μg/ml = 3.34 x 10^6 DPM) in the presence or absence of unlabeled ligand (2.72 to 199.4 μM) for 90 minutes at 37°C. Competitive displacement studies indicate an IC_{50} of 70 μM with a steep displacement curve and >2 x 10^5 binding sites/cell. Following glucan binding, U937 PKA levels were elevated at 6, 12, 20 and 24 h. Maximal PKA activity was observed at 20 h and 100 μg/ml of glucan phosphate. In addition, glucan stimulated IL-8 mRNA expression beginning at 6 h and continuing up to 24 h. We conclude that there are specific binding site(s) on U937 for β-(1→3)-D-glucan phosphate and that ligand-receptor interaction is followed by activation of the PKA signal transduction pathway and expression of IL-8 mRNA.