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IFN-γ/LPS-INDUCED, NO'-MEDIATED CYTOLYSIS IN NON-PHAGOCYTIC CELLS: BIMODAL AND BIPHASIC DEPENDENCE ON GLYCOLYTIC METABOLISM OF GLUCOSE. R. Dijkmans and A. Billiau. Rega Institute, University of Leuven, Belgium.

Mouse embryo fibroblasts (MEF) in culture are killed by treatment with high doses of IFN-γ or by combined low doses of IFN-γ (> 3 U/ml) and LPS (> 10 ng/ml). We demonstrate that: (1) This cytolysis is suicide-like, requiring time (48 h) as well as RNA and protein synthesis (0 - 24 h); (2) The suicidal process is critically dependent on the presence of glucose and its (enhanced) glycolytic metabolism during an early phase (8 - 30 h); (3) The process requires absence of glucose or blockage of glycolysis in a later phase (30 - 48 h); (4) Cell death is prevented by arginine depletion of the medium or by addition of NMMA, an arginine antagonist; (5) Mitochondrial respiration is impaired, and (6) ATP levels are decreased prior to cytolysis.
The data are interpreted to mean that: (a) IFN-γ/LPS treatment triggers synthesis of reactive nitrogen, most likely arginine-derived NO'; (b) Hereby mitochondrial respiratory enzyme systems are damaged, rendering the cell completely dependent on glycolysis for ATP generation and survival; (c) Requirement for early glycolytic metabolism remains enigmatic. IFN-γ/LPS-induced suicidal cytolysis in fibroblasts resembles similar phenomena described in macrophages and may represent a corollary of cellular defense against Gram-negative bacteria.

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FENSPIRIDE PREVENTS LPS-INDUCED LETHAL EFFECT IN MICE
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Fenspiride is a therapeutic agent used in airway and bronchial clinical disorders. Its anti-inflammatory action has been shown (1), despite its lack of NSAID-like or lipoxygenase inhibitory activity (2). Cytokines, especially Il-1 and TNF, have been described as potent polypeptide mediators in the pathogenesis of inflammation (3). The purpose of the present work was to investigate the anti-inflammatory action of fenspiride using an in vivo cytokine-mediated event : LPS-induced endotoxic shock in mice ; this shock is characterized by many organs failure and mortality ; host response to LPS implicate among others, Il-1 and TNF (4). CD1 male mice (23 + 2 g) were injected intravenously with 1 ug LPS, and mortality was observed over 72 hours. Fenspiride used in oral dosing showed a dose-related protective effect within the range 12.5-200 mg/kg : ED50 = 40 mg/kg. In comparison, dexamethasone ED50 was in the range 1-2 mg/kg PO. NSAIDs, i.e. indomethacine at 5 mg/kg, failed to prevent LPS-induced mortality in mice ; therefore this in vivo model is useful to differentiate anti-cytokine agents from NSAIDs. Il-1 and TNF serum levels are increased after LPS administration, and our results suggest that fenspiride may exert its protective effect by an Il-1 or/and TNF inhibition. To verify this hypothesis, experiments are under current investigation.

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(2) Ph. Carré et al., Eur Respir Rev, 1, 79-85, 1991
(3) G. Wakabayashi et al., FASEB J., 5, 338-343, 1991
(4) Ch. A. Dinarello, Adv Imm., 44, 153-203, 1989

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Plasma Interleukin-6, Interleukin-8 and Mortality in Systemic and Localised Gram Negative Infection.

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Raised plasma TNF is generally regarded as a poor prognostic sign in sepsis. There are limited data on IL-6 and the powerful neutrophil chemoattractant IL-8 has not been studied in clinical sepsis. We have performed a longtiudinal study of plasma TNF, IL-6 and IL-8 concentrations in patients with septic (n=10) and localised (n=8) melioidosis due to Pseudomonas pseudomallei. In addition, the level of mRNA for these cytokines was assessed in circulating leucocytes.

Elevated IL-6 concentrations (>1000 pg/ml) were a good indicator of mortality and high IL-8 levels (>100 pg/ml) also indicate poor prognosis. In contrast, 75% of patients who died did not have raised plasma TNF. Levels of IL-6 and IL-8 remained persistently raised in the face of clinical recovery throughout the inpatient period (up to 30 days). Circulating leucocytes contained mRNA for IL-8 but not for the other measured cytokines. These findings have implications for understanding of the pathophysiology of sepsis and for anti-cytokine therapy.

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Interleukin-8 and Malaria

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Malaria remains a major cause of mortality worldwide. Raised plasma TNF concentrations correlate with mortality but do not predict outcome in individual cases. Little is known of the role of other cytokines in the pathophysiology of human malaria.

We have undertaken a longtitudinal study of plasma concentrations of IL-8 and IL-6 in 6 patients with severe *Plasmodium falciparum* malaria in Thailand. Samples were taken on admission, daily for 7 days (by which time parasites had been cleared from the circulation and patients were afebrile) and weekly until discharge from hospital at 1 month. Plasma IL-8 concentrations were elevated to a maximum of 500pg/ml, a level above that typically found in patients with fatal gram negative sepsis. IL-8 plasma levels remained persistently elevated for the 4 week period of the study. Plasma IL-6 was raised at admission (range 11-147 pg/ml) and also remained elevated for the month of the study.

This study demonstrates persistent elevation of plasma IL-8 and IL-6 in patients with severe malaria long after successful clinical treatment. IL-8 is a powerful leukocyte chemoattractant but its role in the pathophysiology of malaria remain to be elucidated.

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A SUPPRESSOR OF THE PHENOTYPIC EXPRESSION OF ras ENCODES LYSYL OXIDASE. Kaylene Kenyon[*], Sara Contente[*], Philip C. Trackman[+], Jin Tang[+], Herbert M. Kagan[+], Robert M. Friedman[*],

[*]Uniformed Services University of the Health Sciences, Bethesda, MD 20814, [+]Boston University School of Medicine, Boston, MA 02118. rrg is a putative tumor suppressor of ras that is expressed at high levels in NIH 3T3, but at very low levels in RS485 (NIH 3T3 transformed by LTR-activated c-Ha-ras). Long-term treatment of RS485 with mouse interferon α/β (IFN) resulted in revertant cells that are stable after IFN treatment was discontinued. Persistent revertant cell lines (PRs) expressed levels of ras p21 and mRNA as high as those of the RS485 line, but were contact inhibited, did not grow in soft agar and did not cause tumors when transplanted into nude mice; rrg expression in PRs is restored to pre-transformation levels. When the PR4 revertant line was transfected with an expression construct carrying a partial rrg cDNA in the antisense orientation, the result was cellular re-transformation. These data suggested that the regulated expression of the rrg gene product forms a part of the pathway of cell transformation by ras: downregulation of rrg correlated with transformation by ras; chronic treatment with IFN resulted in up-regulation of rrg in 1-10% of the treated cells, giving rise to persistent reversion of ras-transformed cells.

A search of GenBank (Release 65.0) with rrg cDNA sequences revealed a match with a 2672 bp cDNA of rat lysyl oxidase. Determinations of lysyl oxidase activity in the culture media of NIH 3T3 and derived cell lines indicated that there was a direct correlation between the lysyl oxidase activity level and rrg mRNA expression level. In addition, lysyl oxidase levels in antisense rrg transfected cell lines correlated with the degree of tumorigenicity previously reported for those lines.

Lysyl oxidase catalyzes the oxidative deamination of peptidyl lysine in elastin and collagen, and can also oxidize lysine in other proteins in vitro. The downregulation of lysyl oxidase expression in transformation and its induction in interferon-mediated reversion of transformed cells suggest the importance of this enzyme in the maintenance of cells in the non-tumorigenic state. It may be that intracellular communication with extracellular crosslinked collagen or elastin is critical, or, it is possible that lysyl oxidase may oxidize other accessible proteins such as membrane-bound receptors capable of transducing signals through ras, or other matrix components, to modulate matrix-cell communication important to the non-transformed phenotype.

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INHIBITION OF TNFα AND IL-1 PRODUCTION FOLLOWING REPEATED ADMINISTRATION OF LPS OR MYCOPLASMA MEMBRANES: R.Gallily and N. Bronstein. The Lautenberg Ctr. for General and Tumor Immunology, The Hebrew U.- Hadassah Med. Sch., Jerusalem.
Our study aimed towards finding whether some of the effects described in LPS-unresponsiveness in vivo are mediated by refractory Mφ. Unresponsiveness was established in vitro using thioglycollate-elicited murine Mφ which were repeatedly incubated with either LPS or Spiroplasma membranes (MQ-1)*. Following Mφ activation for 24h with either one of these agents, the cells remained unresponsive for 3-6 days to a subsequent stimulus by the same activator, as expressed by almost 100% inhibition of TNFα secretion. Pretreatment of Mφ for 4h, was sufficient to render them fully unresponsive for at least 24h. Pretreatment for 24h with either LPS or MQ-1 also diminished IL-1 production following a second stimulus by the same agent, but only partially (about 30%) and for a shorter time (24h). The possible autoregulatory role of prostaglandins of the E series was ruled out by adding indomethacine to the experimental system. As MQ-1 could not stimulate LPS-pretreated Mφ, nor could LPS stimulate MQ-1-pretreated cells for TNFα secretion, we deduce that nonactive state or lack of LPS receptors could not be responsible for Mφ unresponsive state. We are studying whether the unresponsiveness is a transcriptional or post-transcriptional event.
*Sher, et al JNCI 82:1142, 1990.