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IMMUNOHISTOCHEMICAL STUDIES ON THE LOCALIZATION OF ENDOTOXIN (LPS) IN VIVO BY USING HORSESHOE CRAB FACTOR C

K. Uragoh (1), K. Sueishi (1), T. Nakamura (2), S. Iwanaga (2), and K. Tanaka (1). Dept. of Pathology, Fac. of Medicine (1), and Dept. of Biology, Fac. of Science (2), Kyushu univ. Fukuoka-812, Japan.

The localization of LPS in vivo was studied with immunohistochemical method using Ig G against Factor C, which was extracted from hemocyte lysate of horseshoe crab and had the specific affinity to LPS. Organs of rats and guinea pigs were light microscopically investigated at different times after intravenous injection of LPS (E.coli; 0111:B4,026:B6 and salmonella typhosa). Tissues were fixed with buffered formalin and then embedded in paraffin. Deparaffinized sections were incubated with Factor C (1μg/ml) for 1 hr, and then with anti-Factor C Ig G for 1 hr, followed by immunoperoxidase method. The immunohistochemical specificity was examined by absorption of Factor C with LPS, binding competition between Factor C and anti-LPS factor which was extracted from hemocyte lysate of horseshoe crab as well as Factor C or using normal animal tissues and normal Ig G. The immunohistochemical specificity was revealed by these examinations. Immunohistochemically, LPS located predominantly in liver and lung, especially in Kupffer cells and infiltrating monocytes and neutrophils, and aggregated platelets since 5 minutes after intravenous injection of LPS. On the endothelial surface of hepatic sinusoids, glomeruli and pulmonary vessels, LPS was also detected in early period. In addition, LPS was also shown within adrenocortical parenchymal cells, particularly of fascicular zone, later. LPS was not detected 3 days after injection of LPS in liver and lung, but remained during 3 days of observation in adrenocortical parenchymal cells. The present studies revealed that Factor C could be available for immunohistochemical demonstration of LPS in vivo, and reticuloendothelial system, macrophages/monocytes and neutrophils were important as the scavenger cells of LPS and might play a significant role on the development of multiorgan failure in endotoxemia.

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THE DOSE DEPENDENCE OF ENDOTOXIN INDUCED ACTIVATION OF THE PLASMA CONTACT SYSTEM. AN IN VITRO STUDY.

Ø. Røise (1), B. N. Bouma (2), J. O. Stadaas (1) and A. O. Aasen (1). Department of Surgery, Ullevål hospital, University of Oslo, Norway (1) and Department of Hematology, University Hospital Utrecht; Utrecht, The Netherlands (2).

The dose and time dependence of endotoxin induced activation of the plasma contact system was studied in citrated plasma incubated at 37,4 °C. During the first six hours no significant changes were seen. At 12 hours markedly elevated plasma kallikrein (KK) activity was found in plasma tested with the highest doses of endotoxin (2000 and 200 μg/ml). Corresponding to the elevated KK activity reductions of both plasma prekallekrein (PKK) and functional kallekrein inhibition (KKI) were seen. Hageman factor (FXII) values determined immunologically were also reduced parallel to the decreases of PKK and KKI. Changes in high molecular weight kininogen (HMwK) values were seen as well.

In plasma tested with doses of endotoxin lower than 200 μg/ml no sign of activation was seen at 12 hours. At 24 hours, however, increased KK activity and decreased KKI and PKK values were seen in plasma incubated with 20 μg/ml of endotoxin. In test plasma with endotoxin concentrations of 2 and 0,6 μg/ml we found sign of activation in 7 of 8 test tubes using 2 μg/ml and in 2 of 6 test tubes using 0,6 μg/ml. In test plasma with the lowest dose of endotoxin (2,0 μg/ml) and in control plasma no changes in either KK activity, KKI, PKK, FXII or HMwK values were found at 24 hours.

The study shows that endotoxin induced activation of the contact system in citrated plasma is a slow process which is both time and dose dependent.

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ENDOTOXIC LIPID A INDUCES BINDING OF FIBRINOGEN TO HUMAN PLATELETS VIA PROTEIN KINASE C PATHWAY.

Sheila Timmons, Jadwiga Grabarek, and Jack Hawiger. New England Deaconess Hospital and Harvard Medical School, Boston, MA 02215, USA.

Endotoxic Lipid A is the biologically active principle of lipopolysaccharide of Gram-negative bacteria, a most frequent cause of sepsis underlying Disseminated Intravascular Coagulation (DIC) and shock. We have shown that endotoxic Lipid A activates Protein Kinase C in human platelets. Phosphorylation of a 47kDa protein (P47), a marker for Protein Kinase C activation, was observed within the first minute of interaction of Lipid A with platelets. This was accompanied by gradual exposure of the receptor for ^{125}I-labeled fibrinogen (F). Binding of ^{125}I-F was saturable and specific. When Lipid X, a precursor of endotoxic Lipid A and its competitive inhibitor, was used, the binding of ^{125}I-F was blocked with 50% inhibition at a 1:1 stoichiometry between Lipid X and Lipid A. At the same time, phosphorylation of P47 was prevented. Since Lipid X constitutes a "half molecule" of Lipid A, we interpret these results as indicative of competitive blocking of endotoxic Lipid A in terms of Protein Kinase C activation and exposure of platelet receptors for fibrinogen. Binding of fibrinogen is necessary for platelet aggregation and endotoxic Lipid A-induced aggregation was also blocked by Lipid X. Endotoxic Lipid A-induced exposure of fibrinogen receptors via the Protein Kinase C pathway can contribute to involvement of platelets in microcirculatory thrombosis observed in patients with DIC and Gram-negative sepsis.

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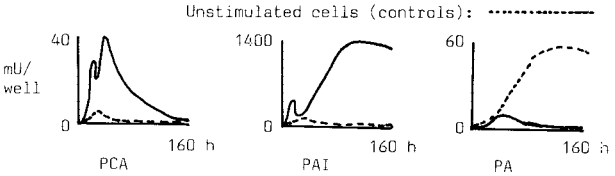
HUMAN PERIPHERAL MONOCYTES STIMULATED WITH ENDOTOXIN IN VITRO SHOWED A FAST PROCOAGULANT ACTIVITY (PCA) ELEVATION AND A LATE ANTIFIBRINOLYTIC RESPONSE.

L.F.Engebretsen (1), P.Kierulf (1), I.Lyberg (2), Å.-B.Andersen (1), M.Gulla (3), G.B.Joø (1). Central Laboratory(1), Department of Surgery(2), and Department of Pathology(3), Ullevål University Hospital, Oslo, NORWAY.

Septicemia causes severe disseminated intravascular coagulation (DIC). Monocytes may contribute to the DIC process through endotoxin stimulated synthesis of PCA. This study was initiated to evaluate the role of the monocytes in DIC further. Blood was taken from healthy persons. Mononuclear leucocytes (30% monocytes) in microtiter wells (25.000 monocytes/well) were added 1 ug/ml endotoxin, and kept in culture from 0 to 160 hours. The cells were harvested, and the PCA (thromboplastin), the fibrinolytic activity (urokinase-PA), and the antifibrinolytic response (plasminogen activator inhibitor-PAI) were measured in the cells. Cell activities were assayed with chromogenic peptide substrates.

Results: Endotoxin stimulated cells : ——— Unstimulated cells (controls): ·····



In the stimulated cells the PCA dominated initially, whilst the antifibrinolytic activity was dramatically elevated after about 40 hours. The fibrinolytic response was minimal compared to the rise in the PAI activity.

In conclusion, the monocytes favoured DIC development during the 160 hours observation period in our experiment through coagulation factor synthesis and hampered fibrinolysis. This support the hypothesis that the monocytes participate in the DIC process.