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ANALYSIS OF LEUKEMIA CELL TISSUE FACTOR BY WESTERN BLOTTING TECHNIQUE. H. Tanaka, N. Narahara, H. Sadakata, K. Andoh, N. Kobayashi and T. Maekawa. The Third Department of Int. Med. Gunma University School of Medicine, Maebashi, Gunma, Japan.

It has been reported that the tissue factor(TF) of monocytes and leukemic leukocytes is one of the trigger substances of disseminated intravascular coagulation(DIC) in leukemia patients. To assess the properties of TF of leukemia cells, their TF was analyzed by the method of Western blotting. Placenta TF was purified using Concanavalin-A affinity chromatography. Briefly, human placenta TF was extracted from placenta acetone powder using Triton X-100 extraction and purified by Concanavalin-A affinity chromatography and SDS-preparative PAGE. The final product of the purified placenta TF-apoprotein exhibited a molecular weight(MW) approximately 46 kD in non-reduced condition and 47 kD in reduced condition and showed single band on analytical SDS-PAGE. Activity of purified placenta apo-TF after relipidation with placenta phospholipid was 4500 units/mg and was 6300 times purified from the starting material. Serum IgG fraction was separated from rabbits after 8 times weekly injections of purified placenta TF. This anti-TF-IgG fraction showed single precipitin line against purified placenta apo-TF and inhibited procoagulant activity of saline extract of the placenta as well as those of homogenates of the endotoxin(LPS)-stimulated Molt-4 cells and U-937 cells. Using this antibody, analysis of TF of cultured leukemia cells(Molt-4 and U-937) and acute promyelocytic leukemia(APL) cells was done by the Western blotting technique as follows: LPS-stimulated Molt-4, U-937 or APL cells were homogenized, delipidated and solubilized with 1% SDS-PAGE. Thereafter, Western blotting was done by the method of Towbin and TF of the blotted protein was immunologically identified. The nitrocellulose paper was soaked with anti-TF-IgG for 18 hours, then peroxidase-conjugated anti-rabbit-goat IgG was used as an indicator of the antibody and o-dianisidine as substrate. In either case of APL cells, LPS-stimulated Molt-4 or U-937, single band which showed procoagulant activity was detected and the MW of the protein was approximately 48 kD in non-reduced condition. These results indicate that TF of all these leukemic leukocytes has common antigenic determinant and equal MW to placenta TF.

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MONOCYTE/MACROPHAGE TISSUE FACTOR: ROLE OF ITS N-GLYCOSYLATED CARBOHYDRATE MOIETY. Shin NAKAMURA. Dept. Biochem., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

Monocytes/macrophages and related cells are known to generate tissue factor (TF), a membrane associated lipid-glycoprotein complex, following activation with LPS or other stimuli. Monkey (M. fuscata) mononuclear leukocytes (MNL, 3 x 10^6/ml) cultured with LPS (1 μg/ml) in FCS-free RPMI medium were stimulated to produce the glycoprotein (TF-Apo). After a lag period of 2 h the TF-Apo production was initiated, and its accumulation reached the plateau after 12 h and then declined to approximately half of the maximum level after 24 h. A time course of the TF activity was strictly in accord with that of the TF-Apo accumulation. Tunicamycin, an antibiotic that blocks the first stage in formation of N-linked oligosaccharides of glycoprotein, affected to reduce the TF expression by 15 to 65 %, when monkey MNL (3 x 10^6/ml) were co-cultured with LPS (1 μg/ml) and the antibiotic (10 to 100 ng/ml) for 10 h. Similar reducing effect of tunicamycin to the TF expression was observed, when RET-1, a macrophage related cell line that generates spontaneouly TF, was cultured with the antibiotic. Interestingly, leupeptin, an inhibitor to trypsin-type proteases including cathepsin B, protected completely the tunicamycin-induced reduction of the TF expression upon its addition to the culture medium at the concentration of 7 μM. Chymostatin, an inhibitor to chymotrypsin-type proteases, also showed the protective effect. These results indicate that TF-Apo of monocytes and RET-1 has N-linked oligosaccharides and that defect of the oligosaccharide chain causes TF-Apo to be susceptible to proteolysis during intracellular processing. Thus, the N-glycosylated carbohydrate moiety of TF-Apo of these macrophage related cells has a roll to stabilize and/or protect it against proteolytic inactivation.

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TISSUE FACTOR OF A HUMAN CELL LINE, RET-1: ITS PRODUCTION, PURIFICATION AND PROPERTIES. Shin NAKAMURA(1), Yukio SUZUKI(2), Takayuki HARADA(3), Shigeru MORIKAWA(3), Shunichiro KAWABATA(4), and Sadaaki IWANAGA(4). Dept. Biochem., Primate Res. Inst., Kyoto Univ., Inuyama(1), Dept. Pharmacol., Okayama Univ. Den. School., Okayama(2), Dept. Pathol., Shimane Med. Univ., Izumo(3), Dept. Biol., Fac. Sci., Kyushu Univ., Fukuoka(4), Japan.

A dendritic cell-like cell line, HLN-Ret-1(RET-1), was found to produce the highest level of tissue factor (TF) among several established cell lines from human lymphoma or leukemia. The TF level expressed by this cell line exceeded 5.6 times that expressed by a monocyte-like cell line, U-937. Unlike other TF producing cell line, i.e. RET-2, HL-60, ML-3, and U-937, the TF expression by RET-1 was spontaneous and unaffected with TPA, PHA, LPS, or MAF. The TF activity of RET-1 was markedly inhibited by Con A as well as that produced by LPS-stimulated monkey monocytes, whereas the TF activity of monkey brain and lung was hardly inhibited by the lectin. Hence, the RET-1 cell lysate solubilized in Triton X-100 was subjected to affinity chromatography on a Con A-Sepharose column, and TF-apoprotein (TF-Apo) was completely bound to the column and eluted with TBS containing 0.15 M α-methylglucoside and 0.1 % Triton X-100. Further purification of this material was performed with combination of FPLC on a DEAE-5PW column and affinity chromatography using a factor VII-Sepharose column. By these methods, TF-Apo preparation with purification-fold of 9,400 and over-all yield of 7 % was obtained. Its apparent molecular weight was estimated to be 120 kDa by gel filtration in TBS containing 0.1 % Triton X-100. SDS-PAGE gave the value of 47 kDa, which was almost compatible with that of TF-Apo from brain or placenta. TF-Apo from the monocytes also bound to the lectin column, suggesting that the apoprotein of these macrophage-related cells has an oligosaccharide chain interacting with the lectin. RET-1 TF-Apo was unstable under acidic condition (<pH 4.0) or in organic solvents such as isopropanol (>18 %) and acetonitrile (>8 %).

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TISSUE FACTOR AND FACTOR VII PRODUCTION BY MURINE BONE MARROW-DERIVED MACROPHAGES: J.W. Shands Jr., T.D. Sunnenberg, and R. Lottenberg. Department of Medicine, University of Florida College of Medicine, Gainesville, FL. U.S.A.

Monocytes and macrophages (macs) make factor VII (VII) and can be induced to make tissue factor (TF), but when during differentiation this capacity is acquired is unknown. We approached this by measuring the synthesis of these factors by bone marrow cells (BMC) during in vitro differentiation into mature macs. BMC were harvested and cultured in RPMI-1640 supplemented with 15mM HEPES buffer, gentamicin, 10% FCS, 15% L-cell conditioned medium, and 2 mM L-glutamine. At intervals, characteristics of macs were measured: size, staining for non-specific esterase, phagocytosis of IgG sensitized RBC, induction of TF by a 6 hr exposure to endotoxin (LPS), and production of VII. The cells increased in mean diameter from 8.5u on d 1 to 15u on d 7 and to 17u on d 15. On d 2-3 of culture few cells were esterase pos, but 51% were phagocytic. By d 7, 95% were esterase pos and phagocytic. After 5 d of culture, macs spontaneously produced small amts of TF as measured by a coupled amidolytic assay. This increased 3 X over the total 15 d of culture. Two d macs did not synthesize TF in response to 1 ug/ml LPS. Responsiveness to LPS began at d 5 and reached a maximum between d 7 and 10 (5X control). The lack of response of 2-3 d macs was not due to suppressor cells, since mixing these with 10 d cells failed to blunt the response of the latter. VII, also measured by a coupled amidolytic assay, was produced on d 2, maximally on d 5, and decreased thereafter. It was not influenced by a 6 hr "induction" with LPS. BMC cultured in the continuous presence of 50 ng/ml LPS were incapable of producing VII and TF. These data suggest that immature macs can synthesize factor VII but not TF and that TF and factor VII synthesis are not coordinately expressed during mac maturation. Culture of BMC in the continuous presence of 50 ng/ml of LPS shut down the production of the coagulant factors by unknown means.