870

THE DISTRIBUTION OF CROSS-LINKED FIBRIN IN HUMAN LUNG CARCINOMA PARALLELS THAT OF ACTIVATED HOST MONONUCLEAR LEUKOCYTES: IMMUNOHISTOLOGIC STUDIES WITH MONOCLONAL ANTIBODIES. F.R. Rickles (1), W.W.Hancock (1), L.Kobzik (2), N.Hogg (3) and C.O'Hara (2). Dept. of Medicine, University of Connecticut Health Center and VA Medical Center, Farmington and Newington, CT (1), Dept. of Pathology, Brigham & Women's Hospital and Harvard Medical School, Boston, MA, USA (2) and King's College, London, England (3).

Previous immunohistologic studies of human lung carcinoma, using polyclonal antibodies to antigens shared between fibrinogen (FGN) and fibrin (FB), showed that FGN/FB were associated with tumor cells. These findings were interpreted as evidence of the presence of tumor-associated procoagulant activity (PCA). We compared the distribution of coagulation-associated proteins in 16 cases of human carcinoma of the lung of varying histologic types, using polyclonal antibodies to FGN/FB and monoclonal antibodies (mAb) to cross-linked fibrin (XL-FB;UC-45), fibronectin (FN;PHM13), factor VIII (vWF:Ag) and a tissue factor-related antigen (TF:RAg; A1-3). Host mononuclear leukocytes were identified using various mAb to T cells and macrophages, and studied for their expression of receptors for interleukin-2 (IL-2R). Positive results are summarized:

	XL-FB+	FGN/FB+	A1-3+	FN+	VWF:Ag+
Tumor cells	1/16	6/16	15/16	0/8	1/10
MNC & stroma	14/16	16/16	16/16	8/8	10/10

In addition, studies of the mononuclear cells adjacent to tumors in 12/12 cases showed the presence of tumor-associated macrophages, 10/11 showed T cells, mainly T8+, and 4/5 showed corresponding expression of IL-2R, suggesting cell activation.

The use of highly specific mAb showed that XL-FB is actually more selectively distributed than is found using polyclonal antisera to FGN/FB, and indeed XL-FB was largely confined to those areas adjacent to tumors which are rich in mononuclear cells. These findings suggest that fibrin deposits in human carcinomas of the lung may be due to development of PCA by activated host mononuclear cells, rather than tumor cells. This lack of XL-FB on tumor cells in spite of A1-3 binding suggests that TF:RAg may not be available on the tumor cell surface for the activation of clotting. Further studies are needed to define the functional capacity of PCA molecules on tumor cells and tumor-associated mononuclear cells in situ.

871

THROMBIN-SPECIFIC SITES OF FIBRINOGEN IN SMALL CELL CARCINOMA OF THE LUNG. L. Zacharski (1), V. Memoli (2) and S. Rousseau (3). Departments of Medicine (1) and Pathology (2), Dartmouth Medical School, and the Veterans Administration Medical Center, White River Jct., VT 05001, U.S.A. (1,3).

Thrombin-generated cleavage sites of human fibrinogen have been identified within the connective tissue stroma adjacent to viable tumor cells in fresh frozen sections of small cell carcinoma of the lung (SCCL) by means of immunohistochemical techniques using mouse monoclonal antibodies (designated alpha and beta) to the N-terminal peptides of the fibrinogen alpha and beta chains (provided by G. Matsueda and E. Haber). Specific connective tissue staining with antibody alpha was diffuse while staining with antibody beta was linear and focal. These results indicate that thrombin is generated in situ in this tumor type. Previous demonstration of an initiator of coagulation together with coagulation factor intermediates associated with viable SCCL tumor cells in situ (Cancer Res. 43:3963, 1983; Blood 66 (Suppl. 1):329, 1985) is consistent with the hypothesis that the tumor cells themselves are responsible for the local thrombin generation. Because favorable effects of anticoagulant therapy with warfarin in SCCL have been demonstrated previously in two randomized clinical trials (J.A.M.A. 145:831, 1981; Proc. Am. Soc. Clin. Oncol. 4:191, 1985), we postulate that local tumor cell-induced thrombin formation may contribute to self-regulated progression of SCCL through formation of a supportive, fibrin-rich connective tissue stroma (N. Engl. J. Med. 315:1650, 1986) and/or stimulation of cell proliferation (e.g. E.M.B.O. J. 4: 2927, 1985; Proc. Natl. Acad. Sci. U.S.A. 83:976, 1986). These results suggest novel treatment strategies for this particular tumor type and justify efforts to identify other tumor types in which similar mechanisms exist.

872

ORAL ANTICOAGULANTS IN BREAST CANCER PATIENTS. A.Sagripanti (1), A.Carpi (2), U.Baicchi (3), A.Nicolini (2), M.Ferdeghini (2), B. Grassi (1). Hematology Service (1), Nuclear Medicine Service (2), Transfusional Centre (3), University of Pisa, Italy.

As the fibrin clot may play a role in the intravascular metastatic spread of breast cancer, we have treated women with advanced breast cancer with oral anticoagulants. After mastectomy, 11 consentient women with postsurgical stage N1 (6 patients), N2 (3 patients) and N3 (2 patients) and without evidence of distant metastasis have been treated with acenocoumarol (INR 2-4,5) for 4-22 months (mean 12 months). All the patients received adjuvant therapy (radiotherapy, polichemiotherapy and/or tamoxifen) in conformity with the classic indications. Fibrinopeptide A plasmatic level, checked in everyone on stable anticoagulation, was always lower than 2 ng/ml (mean 0,78 ng/ml). No major bleeding occurred, except a copious hematuria caused by overdosage. Until now all the 11 women are alive. Two of them stopped anticoagulant, one because of hemorrhagic cystitis, the other because of awareness of visceral metastases; all the other 9 women are being treated now. Three patients (2 N2 and 1 N3) have evidence of visceral metastases; no sign of relapse has been observed by serial instrumental and laboratory examinations in the other 8 women. A control group of 13 patients (9 N2 and 4 N3) was compared with the group of anticoagulated patients in more advanced stages (2 N2 and 3 N3):

	total number	follow-up after mastectomy (mean)	alive	with distant metastases	dead
Anticoagulated patients	5	19 months	5	3	0
Control group	13	18 months	8	5	5

The follow-up of the anticoagulated N1 patients is too brief as yet for comparative evaluation. Our preliminary data seem to indicate an useful role of oral anticoagulants in breast cancer and oblige us to prolong investigation.

873

COAGULATION ACTIVATION AND OCCURRENCE OF HYPOFIBRINOGENEMIA IN PATIENTS WITH ACUTE NONLYMPHOBLASTIC LEUKEMIA. I.Pabinger, K. Lechner, P.Bettelheim, R.Dudczak, W.Hinterberger, C.Korninger, E.Neumann, H.Niessner, I.Schwarzinger. I.Dep.Int.Medicine, University of Vienna, Austria.

122 consecutive adult patients (pts) with acute nonlymphoblastic leukemia (ANLL) were studied for evidence of coagulation activation and occurrence of hypofibrinogenemia. In 16 pts (13%) decreased fibrinogen levels (<1.5g/l) were found. Data on fibrinopeptide A levels (FPA), ethanol gelation test (EGT) and fibrin(ogen) degredation products (FDP) are listed in the table:

	hypofibrinogenemia pts with	pts without
FPA>2.5ng/ml	8/8 (100%)	46/52 (88%)
FPA $\bar{x}$+SD(range)	45+23(20->70)	14+15(1-81)
positive EGT	11/16 (68%)	11/93 (12%)
FDP>10 µg/ml	9/16 (60%)	10/74 (13%)

The occurrence of hypofibrinogenemia was not correlated to the peripheral blast cell count. 10/18 pts with M3 (FAB-classification), 2/46 pts with M2, 2/25 pts with M4 and 2/14 pts with M5 leukemia developed hypofibrinogenemia. Early death occurred in 31% of pts with hypofibrinogenemia (in most pts due to hemorrhage) and only in 10% without (in most pts due to septicemia). No significant difference in remission duration was found between the 2 groups. 48 patients were reinvestigated at the time of recurrence of disease. 5 of 7 patients with and 38 of 41 patients without initial hypofibrinogenemia had a coagulation pattern similar to the one observed at first presentation.
The following conclusions can be drawn:
(1) In most patients with ANLL there is evidence of coagulation activation. (2) Hypofibrinogenemia in patients with ANLL appears to be thrombin mediated. Therefore the term disseminated intravascular coagulation (DIC) may be used. (3) DIC is strongly associated with the subtype M3 (promyelocytic L), but may as well occur in other ANLL subtypes. (4) The presence of DIC seems to be a specific property of certain leukemic clones. This assumption is supported by the coagulation studies during recurrence of ANLL. (5) Patients with low fibrinogen levels are at high risk of early death due to hemorrhagic complications.