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Increased Production of Mononuclear Cell Procoagulant Activity in Hodgkin's Disease*†

P. VIERO,‡ S. CORTELLAZZO,‡ C. CASAROTTO,‡ T. BARBUI,‡ M. COLUCCI§ and N. SEMERARO‖¶

‡*Division of Haematology, Vicenza County Hospital, Vicenza, Italy,* §*Laboratory for Haemostasis and Thrombosis Research, Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62, 20157 Milan, Italy and* ‖*Istituto di Patologia Generale, Medical School, University of Bari, Bari, Italy*

Abstract—*Procoagulant activity of peripheral blood mononuclear leucocytes was studied in 24 consecutive patients with Hodgkin's disease. Mononuclear cells, tested immediately after isolation, expressed very low activity which was, however, somewhat higher than that of cells from a matched control group* ($P = 0.063$). *Procoagulant activity generated by patients' mononuclear cells following stimulation with bacterial endotoxin was significantly higher than that produced by control cells* ($P < 0.01$). *There was no apparent relation between procoagulant activity and pathological staging. The increased capacity of mononuclear phagocytes to produce procoagulant activity might help explain activation of blood coagulation and subsequent fibrin deposition in patients with Hodgkin's disease.*

INTRODUCTION

NORMAL human peripheral blood mononuclear cells, on exposure to bacterial endotoxin and other stimulants (immune complexes, complement proteolytic products, mitogens, etc.), generate a potent procoagulant activity, identified as tissue factor. They are therefore capable of triggering blood coagulation through the extrinsic pathway [1, 2]. It is now generally accepted that the monocyte is the cellular source of procoagulant activity [1, 3, 4]. Recent studies have offered evidence that mononuclear phagocytes might play an important role in the activation of blood coagulation associated with malignant disease by producing procoagulant activity as part of the host immune response to the tumor [5–8].

Recently Harris *et al.* reported the presence of fibrin deposits in the involved lymph nodes and/or spleens of patients with Hodgkin's disease (HD) by using specific immunofluorescence and electron microscopy [9]. This observation, coupled with evidence of increased consumption of platelets and fibrinogen [10] and of elevated plasma levels of fibrinopeptide A [11], clearly indicates that activation of the coagulation system may occur in HD patients. In order to obtain additional information on the possible role of mononuclear phagocytes in the activation of blood coagulation in cancer patients, we investigated the procoagulant response of peripheral blood mononuclear cells in 24 patients with HD before any treatment.

MATERIALS AND METHODS

Patients

Twenty-four consecutive patients with HD, 13 women and 11 men, aged 19–58 yr (mean, 34 yr) and all newly diagnosed, were studied before treatment. Diagnosis of HD was confirmed by histological classification of biopsy material using the Rye modification nomenclature [12]. Fourteen patients had nodular sclerosis, 8 mixed cellularity and 2 lymphocyte predominance histology. Patients were pathologically staged according to the Ann Arbor recommendation [12] as follows: I-A, 2; II-A, 9; II-B, 4; III-A, 3; III-B, 3; IV-A, 2; IV-B, 1.

No severe bacterial infection was present at the time of investigation. Results of screening studies

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¶To whom correspondence and requests for reprints should be addressed at: Istituto di Patologia Generale, Università Policlinico, Piazza G. Cesare, 70124 Bari, Italy.

of the plasma coagulation system, including activated partial thromboplastin time, one-stage prothrombin time and fibrinogen levels, were within the normal range in all patients. A control group consisted of 25 apparently healthy subjects, 12 women and 13 men, aged 20–54 yr (mean, 31 yr).

Isolation of mononuclear cells

Blood anticoagulated with trisodium citrate (0.015 M final concentration) was centrifuged for 15 min at 1300 revs/min and platelet-rich plasma was removed. Mononuclear cells were isolated from the remaining blood by the Ficoll-Hypaque gradient technique as previously described [13]. Final cell suspensions from patients and controls contained more than 97% mononuclear cells and less than 1 platelet per nucleated cell. Monocytes were identified by cytochemical reactivity for alpha-naphthylacetate esterase [14] and T lymphocytes by the monoclonal antibody anti-T3 (OKT3, Ortho Pharmaceutical Corporation, Raritan, NJ) [15, 16]. The patients' and controls' cell preparations contained 10–40% (mean, 24.6%) and 11–30% (mean, 21.8%) monocytes respectively. T lymphocytes, expressed as percent of lymphoid-sized cells, were 57–88% (mean, 73%) in patients and 69–84% (mean, 78%) in controls. Cell viability, assessed by the trypan blue test, was more than 95%. Mononuclear cells of each patient were isolated simultaneously with those of a sex- and age-matched control subject.

Incubation of mononuclear cells and evaluation of procoagulant activity

To study the procoagulant response of mononuclear cells, each patient's and control's cell suspension was adjusted to 0.5×10^9 monocytes/l and, after addition of 20% $BaSO_4$-adsorbed human serum, was mixed with endotoxin (10 μg/ml of *Serratia marcescens* LPS, W., Difco Laboratories, Detroit, MI, U.S.A.) and incubated at 37°C. At predetermined intervals (0 and 4 hr) procoagulant activity generated in the incubation mixture was evaluated by a one-stage recalcification time using the following test system: 0.1 ml mixture, 0.1 ml normal plasma or coagulation factor deficient plasma and 0.1 ml 0.025 M $CaCl_2$. The assay was performed both with intact and disrupted (by repeated freezing and thawing) cells in duplicate in prewarmed plastic tubes. Results were expressed in arbitrary units by comparison of the clotting times of the intact or disrupted cells with a standard curve of clotting times produced by dilutions of a rabbit brain thromboplastin suspension. One thousand units of thromboplastin cause normal plasma to clot in 17 sec. Since procoagulant activity is generated exclusively by monocytes in this system, data are expressed as units/10^5 monocytes.

Normal serum was adsorbed with $BaSO_4$ (100 mg/ml) for 15 min at 37°C with frequent mixing. The procedure was repeated 2–3 times. Adsorbed serum was devoid of factors VII, IX and X (<0.01 U/ml).

Lymphocyte transformation

Lymphocyte transformation was measured as [^{3}H]-thymidine incorporation following *in vitro* stimulation with phytohaemagglutinin (PHA, Wellcome Research Lab., Beckenham, Kent, U.K.) [17]. Triplicate cultures were grown in RPMI 1640 medium supplemented with L-glutamine, gentamycin and 10% heat-inactivated foetal bovine serum in microtitre wells and were stimulated with optimal concentrations of mitogen. Each microtiter well contained 1×10^5 cells. After the cultures were incubated in a 5% CO_2 atmosphere for 72 hr, 0.5 μCi [^{3}H]-thymidine (Radiochemical Centre, Amersham, Bucks, U.K.) was added to each well and incubation was continued for 16 hr. Then the cells were harvested by an automatic device (Titertek cell harvester, Flow Lab., Ayrshire, U.K.) and the radioactivity was determined in a liquid scintillation counter. Results were expressed as the mean incorporation of radioactivity (counts/min + S.E.M.) in PHA-stimulated cultures.

RESULTS

Procoagulant activity was first measured in mononuclear cell suspensions immediately after isolation. Intact cells from either patients or controls had no measurable activity (<0.2 U/10^5 monocytes). Cell disruption resulted in both groups in the expression of very low procoagulant activity (Fig. 1, left side). The latter was somewhat higher in patients ($P = 0.063$).

Figure 1 (right side) shows the procoagulant activity generated by patients' and controls' mononuclear cells after 4 hr of incubation with endotoxin. Procoagulant activity was significantly increased in the patients' group using both intact and disrupted cells ($P < 0.005$ and 0.01 respectively by Student's t test). There was no apparent relation between procoagulant activity and pathological staging. Thus patients with limited (stages I and II) disease had mean values which were not statistically different from those of patients with advanced (stages III and IV) disease (351.9 ± 76.0 vs 451.7 ± 119.2 units/10^5 disrupted monocytes). In addition, procoagulant activity of patients with B symptoms did not differ significantly from that of patients with A symptoms (403.2 ± 143.2 vs 382.2 ± 72.8 units/10^5 disrupted cells).

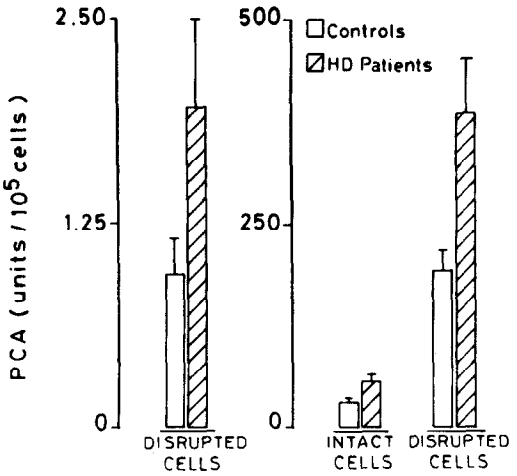

Fig. 1. Procoagulant activity (PCA) of peripheral blood mononuclear cells in 24 patients with HD and in 24 control subjects. Left side: PCA assayed immediately after isolation; right side: PCA generated following endotoxin stimulation. Results are expressed as the mean ± S.E.M.

In all instances PCA was identified as tissue factor since it required factor VII for its expression (Table 1).

It has been suggested that the generation of monocyte procoagulant activity in response to endotoxin and other stimuli results from a cooperative interaction between the monocyte (i.e. the cell actually producing procoagulant activity) and T lymphocytes [1, 4]. The number of T lymphocytes in controls' and patients' mononuclear cell preparations used in this study was not statistically different ($P > 0.1$). To determine the functional status of lymphocytes in our patients we studied lymphocyte transformation in response to PHA. Cell responses to PHA stimulation were significantly lower in patients than in controls (18,277 ± 3520 vs 41,627 ± 2692 counts/min; $P < 0.001$, $n = 19$, by Student's t test).

Table 1. Characterization of mononuclear cell procoagulant activity in 16 patients with HD and in 15 control subjects

Plasma substrate	Range of procoagulant activity (units/10^5 monocytes) Patients	Controls
I Normal	254–571	129–258
II Factor VIII deficient	244–565	135–249
III Factor IX deficient	258–583	126–243
IV Factor VII deficient	3.2–6.5	2.9–4.8

DISCUSSION

Although there is a great deal of information about the impaired lymphocyte function in HD [12], the mononuclear phagocyte system has received less attention and conflicting data have been published about its function. Chemotaxis, Fc receptor activity, helper and suppressor activity on stimulated lymphocytes, phagocytosis and killing may be impaired, normal or even increased [18–22]. A newly recognized function of mononuclear phagocytes is the production of procoagulant tissue factor in response to a variety of stimuli [1, 2]. Edwards and Rickles [1] recently found a marked impairment of MNC procoagulant response in an unusual patient with an immunodeficiency disorder and associated HD. They postulated that a primary monocyte defect may have been at least partially responsible for the depressed generation of procoagulant activity. In contrast, Van Ginkel [23] reported normal or increased monocyte procoagulant activity in 2 patients with HD and in 10 patients with non-Hodgkin's lymphoma. Our findings in a large series of untreated patients with HD suggest that the capacity of monocytes to produce procoagulant activity under stimulus is significantly increased.

Kitahara *et al.* [24] reported that monocyte functional and metabolic activities, measured respectively as chemiluminescence and [^{14}C]-1-glucose utilization upon exposure to ingestible particles, were elevated in lymphomas. Similarly, King *et al.* [25], evaluating glucose metabolic activity of blood monocytes from lymphoma patients, showed enhanced hexose monophosphate shunt. Sheagren *et al.* [26] demonstrated that *in vivo* clearance of radioactive albumin was enhanced and Steigbigel *et al.* [22] observed increased monocyte–macrophage phagocytosis in HD. On the basis of these observations it was suggested that the monocyte–macrophage system in lymphoma patients might be in the activated state [24]. Our experiments would support this concept. In this respect it is noteworthy that mononuclear cells from HD, when tested immediately after isolation, have greater procoagulant activity than control subjects. The precise reason for monocyte activation in HD remains unknown.

It has been suggested that the generation of monocyte procoagulant activity in response to endotoxin and other stimuli results from a cooperative interaction between the monocyte and 'helper activity' of T lymphocytes [1, 27]. To determine the functional status of lymphocytes in our patients we studied mitogen-induced lymphocyte transformation and found, in agreement with most investigators [12], a significant reduction of this function. The increased mononuclear cell procoagulant response in our patients despite evidence of depressed T cell function suggests some considerations on the role

of T lymphocytes in this phenomenon. First production of procoagulant activity may be a T cell-independent function of activated monocytes. Some investigators ([3, 28]; Semeraro *et al.*, unpublished observations) have indeed suggested that normal monocytes are autonomous in the procoagulant response to various stimuli.

Impaired cell-mediated immunity in patients with HD appears to be due, to some extent at least, to intrinsic functional alterations of T lymphocytes [12, 29]. The possibility that a particular property of T cells unrelated to the other immune functions affected in these patients is responsible for the generation of monocyte procoagulant activity should be considered. Alternatively, a population of T cells different from those participating in the other immune responses could be involved.

Our findings may have clinical implications. First, they suggest that the mononuclear cell procoagulant response to various stimuli may represent a useful parameter to assess the monocyte functional status in HD. On the other hand, the increased capacity to produce procoagulant activity might help explain activation of blood coagulation and subsequent fibrin deposition in patients with HD.

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