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Demonstration of HIV-encoded Proteins in Cultured and in Uncultured CD 4 Positive Mononuclear Cells from Hemophilia Patients Employing Monoclonal Antibodies against p 15, p 24, GP 41, GP 120, and Reverse Transcriptase

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In the light of the large percentage of hemophilia patients with antibodies to HIV the identification of a specific virus infection in comparison to HIV antibody negative hemophilia patients has reached crucial importance. The low success rates of direct virus culture techniques together with the as yet low AIDS-disease rate observed in these patients separate these patients from the other main risk groups. Within this context, we studied the expression of CD3, CD4, CD8, and HLA class II antigens on fixed cells after PHA stimulation and Interleukin 2 propagation as well as on untreated blood mononuclear cells from healthy individuals and from hemophilia patients by fluorescence activated flow cytometry. Monoclonal antibodies thought to be specific for p 15, p 24, GP 41, GP 120, and for reverse transcriptase revealed a certain number of positive cells on all defined subpopulations analysed. From cell specimen of HIV antibody positive hemophilia patients cells with specific HIV antigens could be enriched by in vitro cultivation. Importantly the expression of virus-encoded antigens preceedes a cytopathic effect for several days. Current analyses aim at the prognostic relevance of low amounts of such viral HIV proteins selectively detectable by moAbs directed to either p 24, GP 41, GP 120, and RT. The reliability, high sensitivity and monoclonal antibody dependent specificity of this newly developed method for the demonstration of HIV specific proteins make it applicable also for longitudinal surveys of hemophilia patients to assess a potential state of viremia or virus antigen processing in their mononuclear cells.

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EVALUATION OF HIV ANTIGEN AND ANTIBODIES TO p24 AND gp41 IN HEMOPHILIACS. Y. Laurian (1), J.P. Allain (2), F. Verroust (1), M. Leuther (2), T. Lambert (1) and M.J. Larrieu (1). Hemophilia Centers from Kremlin-Bicêtre and La Queue-Lez-Yvelines, France (1) and Abbott Laboratories, North Chicago, Illinois, USA (2).

Two hundred and seventeen hemophiliacs, known to be HIV seropositive for at least one year, were tested for HIV antigen (HIV Ag) and antibodies to p24 (p24 Ab) and gp41 (gp41 Ab) in 1986. HIV Ag was measured using a sandwich-type immunoassay (Abbott Laboratories); this assay predominantly detects core antigen, with a sensitivity of 30 to 50 pg/ml. Antibodies to p24 and gp41 were titrated with separate competitive immunoassays using recombinant antigens (Abbott Laboratories); reciprocal titers were calculated by comparison to a reference curve obtained by serial dilutions of a standard antibody containing sample. Forty-seven of these 217 patients (21.6 %) are HIV Ag positive, with an undetectable or low titer of p24 Ab in most of them. The rest (170) of the patients are HIV antigen negative ; some of them also have a low titer of p24 Ab. All the patients have a high titer of gp41 Ab. The year of seroconversion to HIV is known for 106 patients. Eighty seroconverted between 1983 and 1985 ; 14 of them (17.5 %) are HIV Ag positive (3 had clinical symptoms related to HIV infection, but none developed AIDS) ; 66 are HIV Ag negative (16 developed clinical symptoms, but no AIDS was observed). Twenty six patients seroconverted between 1979 and 1982 ; 12 of them (46 %) are HIV Ag positive ; 14 patients are HIV Ag negative with low (7 cases) or high (7 cases) p24 Ab titers. The table summarizes the clinical data of these 26 patients.

	Number Cases	AIDS	Thrombocytopenia Thrush Zoster	No clinical symptoms
HIV Ag +	12	4	3	5
HIV Ag -/low p24 Ab	7	0	5	2
HIV Ag -/high p24 Ab	7	0	2	5

In conclusion, HIV antigen and antibody titer to p24 may be of predictive value in the definition of lower or higher risk of developing clinical complications related to HIV infection.

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ARACHIDONIC ACID METABOLISM IN PLATELETS STORED FOR FIVE DAYS. J. M. Cesar, J.L. Navarro. Servicio de Hematologia. Hospital Ramon y Cajal. Madrid. Spain.

Arachidonic acid (AA) metabolism has been extensively studied in fresh platelets, but there is little information available for stored platelets . We stored platelets in CLX bags for five days at 22+/-2ºC and, on days 0, 3 and 5, six ml of platelet concentrate were removed from the container and platelets were labeled with (C-14)-AA. Both incorporation and distribution of radiotracer were studied in rest and thrombin stimulated platelets. Total uptake of (C-14)-AA dropped from day 0 to 5 (p 0.01). Distribution on day 0 was similar to fresh platelets. Incorporation of (C-14)-AA on phosphatidylinositol (PI) decayed from 12.4+/-1.5 on day 0, to 7.9+/-0.9 on day 3 (p 0.001), while the percentage attached on phosphatidylserine (PS),increased from 5.3+/-0.9 to 8.8+/-1.5 (p 0.001). There were not any changes from day 3 to 5.

On day 0, 17.7+/-5.2% of radiactivity was released from phospholipids by thrombin. This amount decreased to 7.3+/-2.5% (p 0.01) on day 5. Impairment in breakdown of both PI and phosphatidylcholine (PC) was detected. Generation of phosphatidic acid (PA) by thrombin, decreased from 2.6+/-0.4% of total radiactivity on day 0 to 1.4+/-0.3% on day 3 (p 0.001) and 0.9+/-0.2% on day 5 (p 0.01). We did not find changes in TxB2 and HHT, but HHTE decayed from 7.2+/-2.9% on day 0, to 2.3+/-0.9% on day 5 (p 0.01).

We concluded that both activities of phospholipases A-2 and C are affected by storage.

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IN VITRO NEUTROPHIL ACTIVATION BY PLASMA OF STORED PLATELET CONCENTRATES. L. Muylle , C. Van Brussel , D.R. van Bockstaele and M.E. Peetermans.Department of Hematology and Blood transfusion . University of Antwerp . Belgium.

A previous study showed an effect of storage time of platelet concentrates (PLC) on the frequency and severity of reactions to platelet transfusion.As most of the leukocytes were removed from the PLC prior to transfusion,it was suggested that at least part of the reactions were caused by the transfusion of cell products,accumulated during conservation of the PLC.This idea is supported by the finding of high histamine levels in PLC after 5 days storage.In order to test for the presence of substances causing neutrophil activation,samples from 7 PLC were taken at various storage times and incubated for 30 minutes at 37°C with neutrophilic granulocytes obtained from healthy donors. Binding of a monoclonal antibody specific for a neutrophil-associated membrane glycoprotein (gp 170) was analyzed by indirect immunofluorescence.(CLB 13.9,kindly given by P. Tetteroo).It is known that the expression of gp 170 is enhanced by stimulation of the neutrophil. Fluorescence intensity was quantified by flow cytometry (Ortho cytofluorograf 50-H).Results were expressed as an activation ratio (AR) (ratio of the median fluorescence intensity over that of negative control neutrophils).At day 0 no activation was recorded (AR = 1 $\pm$ 0.09,n = 7). In contrast, the supernatant plasma of PLC stored for 7 days caused an elevated AR in 4/7 PLC (AR = 3.20 $\pm$ 1.20, n = 4).For comparison the AR of the positive control was 3.66 $\pm$ 0.63 (n = 5).In 2 PLC activation was already observed after 1 day storage.The increased expression of gp 170 was not correlated with the histamine level,the lactate dehydrogenase level,the leucocyte or platelet count in the PLC. These preliminary results indicate the presence of factors causing in vitro activation of neutrophils in the supernatant plasma of some stored PLC.Further investigations are needed to confirm these results,to identify the responsible factor(s) and to study the clinical relevance of this finding.