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In vivo and *in vitro* Activation of T-Antigen Receptors on Leukocytes and Platelets[1]

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Abstract. Serological studies on a patient whose red cells are polyagglutinable due to T activation have demonstrated concomitant T activity of the separated leukocytes and platelets. Normal leukocytes and platelets are not T active, but activation can be induced *in vitro* by treatment with neuraminidase or with pneumococcus type III filtrate. Such T-active cells absorb anti-T from *Arachis hypogea* lectin. Tests on different types of separated leukocytes show that both neutrophils and lymphocytes have latent T antigen receptors. Neuraminidase treatment of platelets does not change their ability to promote clot retraction, to aggregate with ADP, or to take up serotonin.

There is evidence that apart from the ABO blood group system [2, 6, 17], a number of antigenic determinants are common to different types of hemopoietic cells. I and i antigens are present on leukocytes [8], the 5b leukocyte antigen is present on red cells [14], and recent investigations have demonstrated that the anti-erythrocyte antibodies, anti-Kx [12], anti-U [10] and anti-Gerbich [11] can be absorbed by neutrophil leukocytes and monocytes from individuals of appropriate genotype. Within each of these last three blood-group systems other antibodies defining red-cell polymorphisms are not absorbed by leukocytes. Normal red cells, leukocytes, and platelets have also been reported to possess latent T antigen receptors. *In vitro* studies have shown that incubation of these cells with neuraminidase induces the ability to absorb anti-T from *Arachis hypogea* lectin [7].

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This report presents studies which demonstrate that *in vivo* T activation of leukocytes and platelets occurs concomitantly with T activation of erythrocytes. Investigation of separated neutrophils, lymphocytes, and platelets has confirmed their susceptibility to *in vitro* T activation and established that such change does not significantly impair the ability of platelets to promote clot retraction or to aggregate with ADP.

Materials and Methods

In vivo *T Activation*

Approximately 100 ml of blood was collected into heparin from a group AB patient with hemolytic anemia secondary to subhepatic abscess, in whom the red cells were T active. *Proteus mirabilis, Klebsiella pneumoniae,* and *Clostridium perfringens* were cultured from the abscess at the time of operative drainage. The patient's red cells were agglutinated by *A. hypogea* lectin, which contains anti-T [3]; but not by *Salvia sclarea* lectin which has anti-Tn specificity, or by *Salvia hormimum* lectin which has both anti-Tn and anti-Cad activity [4]. The heparinized blood was centrifuged at 200 *g* for 15 min at 4 °C and the supernatant plasma containing the platelets removed. The buffy coat was also removed and after resuspension in a small volume of the plasma was recentrifuged using the same parameters. The buffy coat was suspended in Hanks' solution, and anti-A,B serum that had been processed to remove anti-T, was used to agglutinate and sediment the remaining erythrocytes. The leukocyte and platelet suspensions were combined since the volume of each fraction was small. Leukocytes and platelets were prepared from blood of a healthy donor by a similar technique. The leukocyte platelet preparations from the patient and the control were each incubated with *Arachis* lectin at 4 °C for 60 min. The cells were washed four times with saline and an ether eluate prepared [15]. The eluate and, as a control, the supernatant fluid from the final saline wash were tested for activity against the polyagglutinable red cells of the patient as well as neuraminidase-treated and untreated red cells.

In vitro *T Activation*

Buffy coats from 6 U of centrifuged group O donor blood collected into acid-citrate-dextrose solution were separated, pooled, and contaminating red cells in the mixture were eliminated by the addition of dextran. The leukocyte-platelet suspension obtained was separated into its cellular components by centrifugation in a hypaque-dextran discontinuous gradient [16]. This technique yields three fractions containing predominantly neutrophils, lymphocytes, and platelets, respectively. The cell fractions were separated and washed, and an aliquot of the washed donor red cells was taken as a fourth fraction. Each of the four cell fractions was divided into three equal volumes. One volume was incubated with 4 vol of 0.05% papain solution at 37 °C for 15 min and the enzyme subsequently removed by washing. The second portion of each cell fraction was incubated with an equal amount of a known T-activating pneumococcal (type III) filtrate for 30 min at 37 °C, and the cells washed.

The third portion of each cell fraction was kept as an untreated control. Each of the resulting 12-cell suspensions was packed by centrifugation and used to absorb an equal volume of *Arachis* lectin at 12 °C for 60 min. Anti-T activity was assessed by titration against T-active group O red cells and compared with the score obtained following absorption with untreated erythrocytes. These experiments were repeated on a second series of six buffy coat cell preparations to establish the reproducibility of the results. A third series of six buffy coat preparations from group O, D-positive donors were used to absorb anti-D instead of *Arachis* lectin, to confirm that uptake of anti-T by neuraminidase-treated leukocytes and platelets is a specific phenomenon, and to ensure that the leukocyte-platelet preparations were free of contaminating red cells or red cell stroma.

In a further study, leukocyte preparations were prepared from the blood of four donors, and pooled. Lymphocytes were isolated by hypaque-dextran discontinuous gradient centrifugation and the separated cells divided into two aliquots. One was incubated with neuraminidase at a concentration of 50 U/ml for 30 min at 37 °C, washed, and checked for T activity by using some of the cells in an absorption-elution test with *Arachis* lectin. The second part of the lymphocyte preparation was left untreated and used in a parallel *Arachis* absorption experiment as a control.

Platelet Function Tests

Blood of ten nonfasting donors who, as far as could be ascertained, had taken no medication in the previous 7 days, was collected into acid-citrate-dextrose solution. The platelets were separated by differential centrifugation in a hypaque-dextran gradient and pooled. After washing in buffered saline, the cell suspension was divided into two aliquots. One was retained as an untreated control, while the other was incubated with neuraminidase at a concentration of 50 U/ml for 30 min at 37 °C. Enzyme treatment was arrested by further washing and a portion of the cells checked for T activation by an absorption-elution test with *Arachis* lectin.

Platelet ^{14}C-serotonin uptake and release of the enzyme-treated and control cells was investigated by the method of ZUCKER and PETERSON [18]. The aggregative response of the platelets to added epinephrine and adenosine diphosphate was measured in a chronolog platelet aggregometer. To determine clot retracting capability, treated and untreated platelets were added to platelet-poor heterologous plasma to give a final concentration of 250,000/μl. 5 U of thrombin were added to 2-ml volumes of the test preparations and clot retraction measured by the method of BENTHAUS [1].

Results

In vivo *T Activation*

Microscopic examination of the patient's leukocyte-platelet preparation revealed many neutrophils, lymphocytes, monocytes, and platelets, as well as an occasional red cell. The proportion of red cells to leukocytes

Table I. Reactivity of *Arachis hypogea* lectin recovered after absorption and elution using a leukocyte-platelet preparation (4 °C, 15 min)

Source of leukocyte-platelet preparation	Red cells used to test eluate		
	control		T-active patient
	untreated	neuraminidase	
T-active patient	0	5	7
Control	0	0	0

Table II. Titration scores [9] of *Arachis hypogea* lectin after absorption with enzyme-treated or untreated separated cell fractions

Absorbing cells	Untreated	Treatment of absorbing cells	
		papain	bacterial filtrate
Neutrophils	15	12	5
Lymphocytes	14	20	0
Platelets	19	23	0
Red cells	20	21	0

Table III. Results of absorbing *Arachis hypogea* lectin with neuraminidase-treated and untreated lymphocytes

	Reciprocals of dilutions of *Arachis* lectin							Score [9]
	2	4	8	16	32	64	128	
Before absorption	12	12	12	7	6	3	0	52
After absorption with								
Untreated lymphocytes	12	12	12	5	4	2	0	47
Neuraminidase-treated lymphocytes	3	0	0	0	0	0	0	3

was determined by direct count. Based on this ratio, sufficient T-active red cells from the patient were added to the normal control preparation to give the same final proportion. The patient and control leukocyte-platelet preparations were then used in absorption-elution experiments with *Arachis* anti-T lectin. The results shown in table I, establish that the leukocyte-platelet preparation from the patient's blood had strong absorptive capacity for anti-T, while the control preparation had none. In both cases, efficiency of the final cell washing procedure was demonstrated by testing the last wash solution for anti-T activity.

In vitro *T Activation*

Microscopic examination of the separated cellular components of the buffy coat preparations revealed the platelet fraction and the neutrophil fraction to be essentially pure, and the lymphocyte fraction to contain predominantly lymphocytes with small numbers of monocytes. Following treatment of the neutrophil preparation with papain, the cells became difficult to separate and prolonged centrifugation was necessary. The results of absorbing *Arachis* lectin with aliquots of the different cell fractions that had been treated with papain, with T-activating pneumococcus filtrate, or left untreated, are shown in table II. Absorption with papain-treated or untreated cells did not reduce the anti-T activity of the *Arachis* lectin. Absorption with platelet and both leukocyte pneumococcus filtrate-treated preparations caused a significant decrease in anti-T activity. None of the leukocyte or platelet preparations absorbed anti-D, thus confirming that these cell fractions were not contaminated with red cells. All of the red cell preparations absorbed anti-D and as expected the red cells exposed to papain or T-activating filtrate removed more antibody than the untreated cells. Neuraminidase-treated normal lymphocytes showed strong absorptive capacity for lectin anti-T but untreated cells had no such capability (table III).

Platelet Function Tests

Both neuraminidase-treated and untreated platelets showed normal uptake of ^{14}C-serotonin, but in neither case was it released by the addition of adenosine diphosphate (ADP) or epinephrine. Both platelet preparations gave the same low normal results in tests for aggregation following addition of ADP. Neuraminidase treatment did not affect ability of the platelets to induce clot retraction and both cell preparations gave the same normal result.

Discussion

In vivo polyagglutinability caused by T activation has long been recognized as an enzymatically induced modification of red cells. It is, however, apparent that the phenomenon is in reality one of more generalized desialization that exposes latent antigen receptors in red cells, leukocytes, and platelets. It appears that the terminal neuraminyl groups which are characteristic of vertebrate blood and tissue cells [13] are attached to a common fundamental membrane structure, which, following cleavage of neuraminyl groups by neuraminidase, interacts specifically with anti-T of *Arachis* lectin.

Tn receptors have also been demonstrated on all cellular hemopoietic elements [7]. While patients whose red cells are polyagglutinable due to Tn activation frequently exhibit leukopenia and thrombocytopenia [5], similar changes are not typical of *in vivo* polyagglutination caused by T activation. Furthermore, while the technical procedures used in isolating platelets for *in vitro* T activation impaired their functional activity in some of the studies, the T-active platelets showed no more deficiency than did the untreated control platelets. It appears possible, therefore, that the generalized hematologic *in vivo* antigenic changes that are seen in T activation may not affect cell functional activities.

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