Lymphocyte Transformation Induced by Autologous Platelets in a Case of Thrombocytopenic Purpura

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In a case of chronic thrombocytopenic purpura, significant lymphocyte transformation could be induced with autologous platelets as a stimulant. The reaction was not due to serum factors since it occurred when culture medium was supplemented either with autologous or pooled human serum. Controls excluded nonspecific lymphocyte transformation. Modulation of normal platelet antigens by viral or chemical factors or adsorption of such factors on the platelet membrane could account for the observed reactions. However, since other autoantibodies were demonstrated, the hypothesis of altered cellular "self-recognition" mechanisms is tentatively proposed as an alternative explanation for this observation.

Since the original demonstration that phytohemagglutinin can induce DNA-synthesis, blastic transformation and mitosis of small lymphocytes, it has been recognized that several other factors can act as lymphocyte stimulants. Of these, antigens to which the lymphocytes have been sensitized are among the most efficient, but previous contact with the stimulating agent is not an absolute condition for the in vitro transformation of small lymphocytes.

Some forms of thrombocytopenic purpura have been explained by an immunological reaction directed against autologous platelets, and numerous reports have dealt with the detection of platelet-directed circulating antibodies (for summary, see references 9 and 10). It seemed, therefore, logical to test this hypothesis by means of lymphocyte transformation. The results of such
an experiment in a patient with thrombocytopenia, but otherwise hematologically normal, are reported herein.

CASE REPORT

A 73-year-old woman was first seen at this Institute in November 1965 for purpura. Family and previous history were unremarkable. Physical examination was normal except for the presence of purpura on both arms and chest. The hemoglobin was 13.8 Gm. per cent, the total white blood cell count 4400/cu. mm. with a normal differential count and platelets 2000/cu. mm. A bone marrow aspiration showed the presence of large numbers of normal megakaryocytes. Search for L.E. cells was negative and the usual blood chemical determinations completely normal. Oral Triamcinolone (Kenakort) 48 mg./day was started on December 2, 1965 and continued until January 1969. With this regimen, the platelet count rapidly became normal, but each attempt to reduce the drug dosage was promptly followed by a significant decrease in platelet count.

Since multiple side effects of this prolonged corticosteroid treatment appeared (diabetes mellitus, hypertension with congestive heart failure and generalized osteoporosis with bone pain), a splenectomy was performed in January 1969. Pathological findings in the spleen were consistent with the diagnosis of idiopathic thrombocytopenic purpura; no other abnormalities, such as lupus erythematosus, were seen.

Following this treatment, normal platelet values were adequately maintained without steroids except for a transitory episode of thrombocytopenia without clinical manifestations in July 1969.

In September 1969, the patient was readmitted for generalized purpura and melena. Physical examination was within normal limits except for the hemorrhagic lesions. The platelet count was 100/cu. mm, hemoglobin 6.6 Gm. per cent, white cell count 14,500/cu. mm.

No accessory spleen was demonstrated by isotope scanning. A total of 2500 ml. of whole fresh blood was administered. During the following days, the platelet count rose to normal values. Since hemorrhagic diverticulitis was demonstrated by sigmoidoscopy and barium enema, a low residue diet and atropine sulfate (0.25 mg. x 4/day, p. o.). were started on October 3. Iron was given as ferrous sulfate (250 mg. x 4/day, p. o.).

Changes in the platelet count during the period when the patient was studied are given in Fig. 1. The steroid treatment was stopped in December 1969 without a fall in platelet count.

During the course of her illness, this patient took several medications including aspirin, penicillin and tetracyclines, barbiturates, phenylbutazone and several diuretics of the thiazide class. However, most of them were administered on several occasions and none of them was clearly associated with a definite fall of the platelet count. At the time of study, there was no clinical evidence of viral disease, and a search for cold agglutinins was negative. No rise in the titer of antigens against common viruses could be demonstrated.

During the course of the disease, intermittent positive results were obtained for the indirect Coomb’s test, antithyroid antibodies, Sia test and platelet agglutinins (titer 1/1 to 1/4). The complement titer was lowered at the time of the study.

MATERIALS AND METHODS

Platelets of the patient (P) and of a normal control donor (P') were isolated by the technique of Klein.11 The final platelet suspension was in approximately 15 ml. of autologous plasma and will be referred to as “platelets.”

To obtain lymphocyte suspensions, Plasmagel (Lab. R. Belon, Paris) was added in a final concentration of 1 ml./10 ml. of cell suspension to the cell precipitate from the first centrifugation in isolating platelets. The mixture was allowed to sediment at 37°C. The leukocyte-rich supernatant was transferred to a cotton-wool column and incubated at 37°C for 45 minutes. Elution of the column with TC 199 culture medium yielded a lymphocyte suspension containing less than 4 per cent polymorphonuclear cells and less than 5 per cent platelets. This suspension was concentrated by centrifugation and counted...
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Fig. 1.—Evolution of platelet count during the period of study.

in a hemocytometer. Cell viability was judged by the exclusion of trypan blue on several samples and was $96 \pm 5$ per cent.

Mixed cell cultures (MCC) were prepared by the technique of Bach adapted for the use of platelets as a mitogenic agent. The culture medium was TC 199 (Difco) supplemented with 20 per cent autologous or pooled human serum as indicated. Antibiotics were omitted. The cell mixtures were incubated at $37^\circ\text{C}$ in 2.5 ml. of the medium in tightly sealed siliconized test tubes.

Each tube contained $1 \times 10^6$ responding lymphocytes. Phytohemagglutinin (PHA-P, Difco diluted 1:100 in physiological salt solution), stimulating lymphocytes ($1 \times 10^6$) or platelets were added to 0.1 ml. volumes. In order to obtain one-way stimulation in the mixed lymphocyte cultures (MLC), stimulating lymphocytes were preincubated with Mitomycin C (0.05 mg./ml.) at $37^\circ\text{C}$ for 30 minutes and washed twice in TC 199 thereafter.

Twelve hours before the end of the incubation 4 $\mu$Ci. of tritiated thymidine (3H-TdR,
specific activity 1.9 Ci./mM, Radiochemical Centre, Amersham) in 0.1 ml. were added to
the test tubes. PHA cultures were incubated for 72 hours, MCC for 144 hours. To stop the
reaction, the cells were spun down, the supernatant removed and the cells precipitated with
5 per cent cold trichloroacetic acid. The radioactivity of the precipitate was determined in a
Philips liquid scintillation counter as described previously. All experiments were performed in duplicate and the results are expressed as mean counts per minute (cpm) for each combination after correction for quenching by the channel ratio method.

Additional cultures were prepared for morphological studies. One hour before the end
of the incubation period 1 µCi 3H-TdR per ml. medium was added to the tubes. The cells
were washed twice in TC 199. The smears from the cell pellets were air-dried, fixed in
methanol-acetic acid (3:1) for 2 hours at 4°C and processed for autoradiography with
Kodak NTB2 liquid emulsion. After 70 days incubation in the dark, the smears were
stained with May-Grünwald-Giemsa.

RESULTS

The results of isotope incorporation are given in Figs. 2 and 3. It can be
seen from Fig. 2 that the greatest rate of 3H-TdR incorporation occurred
when the patient's (L) or normal control donor lymphocytes (L') were stimu-
lated with PHA. Both donor's lymphocytes were reactive in one-way MLC. The
patient's lymphocytes could be stimulated in MCC with either allogeneic or
autologous platelets. Control lymphocytes could be stimulated with allogeneic
platelets, but failed to transform in the presence of autologous platelets. No
significant differences in the response to the various mitogenic agents occurred

![Fig. 2.—Incorporation of 3H-TdR into cellular DNA for different test combinations. Values are expressed as mean corrected counts per minute (cpm); the first symbol indicates the type of responding cells, the second symbol gives the mitogenic agent. L, patient's lymphocytes; Lm, idem, mitomycin pretreated (see text); L', normal donor's lymphocytes; L'm, idem, mitomycin pretreated; P, patient's platelets; P', normal donor's platelets; PHA, phytohemagglutinin; N, nonincorporating controls.](image-url)
Fig. 3.—Effect of autologous and pooled human serum on incorporation isotope into cellular DNA. Symbols are as in Fig. 2; left columns, with autologous serum; right columns, with pooled human serum. The differences are not significant.

when autologous serum was substituted for pooled human serum in the cultures with L as responding cells (Fig. 3).

Neither platelets alone nor unstimulated lymphocytes showed any significant incorporation of 3H-TdR under the experimental test conditions. CPM for these combinations are referred to in the figures as “nonincorporating controls” (N).

The morphological studies gave essentially the same results as those obtained by the incorporation of 3H-TdR for the various cell combinations. In some smears, an occasional platelet was seen amidst lymphocytes showing the various stages of blastic transformation.

DISCUSSION

The diagnosis of idiopathic thrombocytopenic purpura (ITP) is usually made by exclusion of toxic and infectious origins. This patient had several episodes of severe thrombocytopenia for which no drug-related or infectious origin could be demonstrated. There was no underlying disease that could have been responsible for a secondary thrombocytopenia. This, together with the chronic cyclic course and the pathological findings in the spleen, suggest that this patient suffered from ITP. The therapeutic effectiveness of both corticosteroids and splenectomy could be invoked as additional evidence in favor of this diagnosis.

When a chemical compound can be incriminated as responsible for thrombocytopenia, the pathogenesis is often of an immunological nature,
and thrombocytopenia can be reproduced in vivo or platelet aggregation may be demonstrated in vitro with the causative agent. In contrast, ITP has been shown on several occasions to be caused by an immunological mechanism. Indeed blood or serum from these patients, obtained during episodes of active ITP, can induce marked thrombocytopenia in normal volunteers or relapses when it is autotransfused during periods of remission. The factor responsible for this is present in 7S gamma globulin. In addition, several methods have been developed to assess platelet antibodies in vitro, but most of them failed to yield unequivocal results in ITP.

Lymphocyte transformation is commonly considered to be the in vitro counterpart of some early step of antigen recognition in vivo. It can be induced by several mitogens including membrane-located (transplantation) antigens. The present results of in vitro lymphocyte transformation, induced by autologous platelets at a time when thrombocytopenia appeared in vivo, strongly suggests that in the case under discussion an immunological cell-mediated mechanism was responsible for the thrombocytopenia.

Modulation of the normal platelet membrane antigens or induction of new antigenic determinant(s) by a viral or chemical factor could cause lymphocyte transformation in a manner similar to that observed in mixed tumor cell-lymphocyte cultures. Adsorption on the platelets of similar antigenic factors without alteration of the normal platelet antigenicity could also explain the present findings. Since the reactions were essentially the same whether autologous or pooled human serum was added to the culture medium, it seems unlikely that a serum factor was involved in the lymphocyte stimulation. However, since the “stimulating” platelets were used without previous washings, it cannot be excluded that a complex formed by platelet and antiplatelet antibodies acted as the stimulant. Such antigen-antibody complexes are able to induce lymphocyte transformation.

In view of the intermittent positive Coombs’ test, the presence of anti-thyroid and anti-platelet autoantibodies and the low complement level at the time of study, one could invoke an alternative hypothesis of altered immune recognition mechanisms leading to a state of lymphocyte hypersensitivity to autologous antigens as in other autoimmune diseases. If this hypothesis is true, the basic defect of the disease presented by this patient, and possibly in other cases of thrombocytopenic purpura, would lie not in the platelets but in the lymphocytes. The cyclic course of our patient’s illness is difficult to explain then, but normal platelet values could be maintained even in the presence of sensitized lymphocytes by the same mechanism that permits antigenic tumors to grow in vivo in the presence of in vitro cytotoxic lymphocytes. These mechanisms were recently reviewed by Hellström.

**CONCLUSION**

The possibility that cell-mediated immune mechanisms may be involved in thrombocytopenic purpura needs further investigation and should be confirmed by additional patient studies. The time at which such studies should be done is difficult to assess, but the present observations suggest that the optimum time may be as thrombocytopenia develops in vivo.
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REFERENCES


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