Megakaryocytopenia in Experimentally Induced Immune Thrombocytopenia

By Zoran Rolovic, Mario Baldini and William Dameshek

It has often been suggested that in immune thrombocytopenia, platelet antibody may not only cause destruction of circulating platelets but also exert an injurious effect on the megakaryocytes of the bone marrow. This suggestion was made mostly on the basis of the well-known morphologic alterations observed in the megakaryocytes of patients with idiopathic thrombocytopenic purpura (I.T.P.), and in experimentally induced immune thrombocytopenia both in animals and humans.\(^1\)\(^-\)\(^4\) Evidence was subsequently presented, using the fluorescent antibody technique, that platelet antibody could often be found in the bone marrow, selectively attached to the surface of the megakaryocytes.\(^5\)\(^-\)\(^9\)

The hypothesis that in immune thrombocytopenia an antibody-mediated injury to the megakaryocytes can, in part, be responsible for the low platelet count seems acceptable, especially in view of the lack of evidence for increased platelet production in I.T.P.\(^11\)\(^-\)\(^12\) However, opposing points of view still exist. Thus, the morphologic alterations of the megakaryocytes seen in immune thrombocytopenia have been interpreted as an expression of immaturity of these cells which have become abundant in an attempt to compensate for the increased rate of peripheral platelet destruction.\(^13\) Furthermore, from data obtained with platelet survival studies in patients with I.T.P., recent investigators have concluded that in immune thrombocytopenia the marrow megakaryocytes are indeed capable of increased platelet production which would reach two to eight times the normal\(^14\)\(^-\)\(^18\) or even ten times the normal, if triggered by a profoundly reduced platelet level together with active bleeding.\(^15\)

Approaches thus far utilized to define the effect of platelet antibody on the megakaryocytes in immune thrombocytopenia have all been indirect. In the present study, this problem was explored by the use of tritiated thymidine (\(^3\)HTdR) as a cell label for bone marrow autoradiography in an attempt to obtain direct information on megakaryocytopenia during prolonged thrombocytopenia experimentally produced in rats by a potent, heteroimmune anti-

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Fig. 1.—Changes of circulating platelet counts in rats presented in upper portion of chart. Open circle represents mean value of 30 rats. Black dots within enclosed areas represent values of individual platelet counts in rats injected with $^3$HTdR. Arrows in upper part of panel indicate time at which platelet antiserum was injected or thrombocytopheresis performed or adsorbed antiserum given. In lower part of chart, hematocrit values reported. Points (solid circle and bracket) represent mean value ± 1 SD of 30 rats, except for values on 4th, 5th, 6th day which were derived from rats injected with $^3$HTdR.

platelet serum. Two control models were also used; one of rats in which the platelet count was maintained at low levels by repeated thrombocytophereses, and another of the rats injected with a platelet antiserum previously adsorbed with rat platelets.

**MATERIALS AND METHODS**

Female Sprague–Dawley rats* weighing 225–248 Gm. were used in this study. They were divided into three experimental groups (Fig. 1; upper part): (a) the "immune thrombocytopenia" group in which the platelet counts were maintained at lower than normal level by repeated intraperitoneal injections of potent rabbit antirat-platelet serum; (b) the "thrombocytophoresis" group in which the platelet counts were continuously reduced by repeated exchange transfusions with platelet-poor blood; (c) the "adsorbed antiserum" group in which the rats were repeatedly injected with platelet-adsorbed antirat-
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platelet serum. Each group of rats consisted of 30 animals. On the fourth day of the experiment, each rat was injected with a single dose of ³HThD.R, 0.75 μCi/Gm. body weight via a tail vein. At chosen intervals thereafter, the rats were serially sacrificed. Three rats from each group were sacrificed at 30 minutes and at 2, 8, 12, 18, 24, 36, 48, 54 and 60 hours after the injection of ³HThD.R. Femoral bone marrow smears and sections were made and autoradiographs were prepared.

Platelet counts* at the time at which the rats were sacrificed ranged from 160,000 to 360,000/mm.³ (mean 250,000 mm.³) in the "immune thrombocytopenia" group, while in the "thrombocytopenia" group they ranged from 300,000/mm.³ to 590,000/mm.³ (mean 376,000/mm.³), and in the "adsorbed antiserum" group they were between 870,000 and 985,000/mm.³ (mean 925,000 mm.³). Values for the immune thrombocytopenia group were lower by 73 per cent, and for the thrombocytopenia group by 59 per cent than the respective initial values. No change from the initial value was noticed in the adsorbed antiserum group. Microhematocrit determinations† were taken in parallel with each platelet count (Fig. 1; lower part). These values did not significantly differ from the initial ones.

EXPERIMENTAL DETAILS

Preparation of Antirat-platelet Serum

The antirat-platelet serum was prepared in the rabbit according to classical descriptions of earlier investigators.17-19 Several technical refinements more recently reported,20-21 were adopted in order to obtain an antirat-platelet serum with a high degree of potency as well as specificity. Four albino rabbits were immunized with rat platelets which had been carefully separated and were free of leukocytes and erythrocytes. After seven successive washes with saline, the platelets were disrupted by sonication. The suspension was then injected in the foot pad of the rabbits after mixing with an equal volume of complete Freund's adjuvant. Ten injections were given during a six-week period. A total of about 2 X 10² platelets were used for each rabbit. On the seventh week, the serum was collected from the rabbit blood. After inactivation of thrombin with a one-twentieth volume of a 1.34 per cent solution of sodium oxalate, the serum was treated with barium sulphate (200 mg. per ml.) and heated to 56°C for 30 minutes. The serum was also subjected to successive adsorptions with rat red cells, endothelial scrapings from rat aorta and rat kidney homogenate. After the last adsorption, the globulin fraction of the serum was separated by treatment with sodium sulphate. It was then dialyzed against normal saline. The globulin fraction was subdivided into small aliquots and stored at -20°C until use.

When the serum was tested by immunodiffusion27 against rat platelet sonicate, two precipitin lines developed. No precipitin line developed in the presence of red cell ghosts, kidney homogenate, or when the antirat-platelet serum had been adsorbed with washed rat platelets.

Production of "Stable" Immune Thrombocytopenia in the Rats

The following procedure was used in order to produce a relatively stable thrombocytopenia in the rats (Fig. 1). On the first day, the rats were injected intraperitoneally with 0.3 ml. of the globulin fraction of the antirat-platelet serum. The platelet count decreased sharply during two to four hours to 40,000-65,000 per mm.³ and remained practically unchanged for 19 to 20 hours after which it started to increase slowly, but never reached the level of 200,000 per mm.³ at the end of 24 hours. During the three subsequent days, rats were injected daily with 0.1-0.15 ml. of the globulin fraction of the antirat-platelet serum, and for two more days with 0.2 ml. of the same material.

†Schwarz BioResearch Inc., New York, 1.9 Ci/mM., diluted with sterile saline to 500 μCi/ml.

*Platelet counts were determined by phase microscopy16 of blood collected from the tip of the rat tail.

†Adams-Clay microcentrifuge, New York, N.Y.
Repeated thrombocytophereses were performed in a second group of 30 rats in order to study control animals with a nonimmune type of thrombocytopenia. This was done by repeated exchange transfusions with platelet-poor blood. The technique utilized was a combination of that of Arnould et al., Matter et al., and Brodish and Long. The method will be reported elsewhere in more detail, but fundamentals were as follows. An indwelling plastic catheter was placed and fixed securely into the right internal jugular vein and, through the muscles of the neck, brought to the skin surface and tied to it. During the period of the experiment, patency of the catheter was maintained by the heparin and the buffered saline solution in the tubing. Beginning on the fifth day of the experiment, the animals received daily injections of heparin intraperitoneally because of the increasing tendency of the jugular catheter to become occluded. Exchange transfusions were performed daily under ether anesthesia, and each time approximately 2.5–3 times the rat blood volume was exchanged with platelet-poor blood from donor rats. The platelets removed during the first day numbered 11–14 $\times 10^9$ per animal. During the next three days, 3–6 $\times 10^9$ platelets per day were removed from each animal. It should, however, be noted that in spite of the fact that during the fifth and sixth day 7–10 $\times 10^9$ platelets per day were removed, platelet counts showed tendency to increase. Hematocrit levels in this group were often slightly lower than normal, but not significantly so (Fig. 1).

In a group of five other rats, the plastic catheter was placed in the jugular vein and heparin was infused repeatedly in the same quantities used in the animals described above, but no thrombocytopenia was performed. In 10 additional rats exchange transfusions were performed with fresh blood from donor animals (containing normal amounts of platelets), with the same frequency as in the animals in which thrombocytopenia was produced. One rat from the first group and two rats from the second group were sacrificed at 30 minutes, 12, 18, 24 and 36 hours after a $^3$HThdR injection was given. Platelet counts were within normal limits and the pattern of megakaryocytopoiesis in these animals was identical to that seen in untreated, normal animals. Megakaryocytopoiesis in normal rats of the strain used in the present work was studied by us in a parallel work published elsewhere.

### Adsorbed Antiserum Group

Thirty rats were injected through the same route, and with the same quantities of globulin fraction of an antiserum which had been repeatedly adsorbed with washed rat platelets. The platelets used for each adsorption were 1–2 $\times 10^9$ per ml. of serum. Each adsorption was carried out at 37°C for 30 minutes and was repeated until the serum produced no in vitro agglutination of rat platelets and developed no precipitin line on immunodiffusion with rat platelet sonicate. The rats injected with the globulin fraction of the adsorbed antiserum always maintained their platelet counts within normal limits. The pattern of megakaryocytopoiesis in these animals was identical to that seen in normal animals.

### Autoradiography of the Bone Marrow

The technical procedure for bone marrow autoradiography and the principles for megakaryocyte classification were, in general, according to Feinendegen et al. and Ebbe and Stohlman. Only personal variations will be reported here. All autoradiographs were made with Kodak NTB2 nuclear track emulsion. They were exposed for 30 days at +4°C. After development, they were stained with Wright’s stain buffered at pH 6.8, then with Giemsa stain buffered at pH 5.6.

Megakaryocytes were classified on the basis of their morphological characteristics into

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*Sorensen’s phosphate buffer made of 1/15 molar monopotassium phosphate, and 1/15 molar disodium phosphate.*
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stages I, II, and III, as proposed previously by ourselves and others.\textsuperscript{1,32,33} Stage-I megakaryocytes were the earliest recognizable immature forms having an intensely basophilic cytoplasm without granulations. These cells were generally the smallest among the megakaryocytes. Their nucleus occupied most of the cell and was not lobulated. The chromatin was granular. In stage II, the size of the cells was generally larger and the nuclear–cytoplasmic ratio was lower. The nucleus was usually lobulated and had granular chromatin. The cytoplasm was less intensely basophilic and had a foamy appearance. Cells of stage II had a few azurophilic granules in one or two rose-colored localized areas of the cytoplasm. Stage-III megakaryocytes were the most differentiated forms, having a slightly bluish cytoplasm with diffuse azurophilic granulations, at times in groups. The nuclear–cytoplasmic ratio was still lower and the nucleus was either single or multiple or segmented with denser and coarser chromatin. The largest and most mature megakaryocytes with pyknotic nuclei and light cytoplasm breaking into platelets were also included in this group.

In this study, particular attention was focused upon signs of cytoplasmic maturation (mainly appearance of granulation, as originally emphasized by Dameshek and Miller)\textsuperscript{1} as a distinguishing feature among the three maturation stages. Less importance was given to the size of the cells and the degree of nuclear segmentation. “Naked” nuclei were included in stage III only if they had a complete or partial rim of cytoplasm; they were otherwise discarded.

Megakaryocyte differential counts and calculation of the labeling index were done by counting 1000 cells for each rat. By evaluating 1000 cells, according to our data,\textsuperscript{34} scattering of values of megakaryocyte differential as well as of labeling index was much less pronounced than when smaller numbers of cells were evaluated. Background was calculated by a modification of the Stilstrom’s technique.\textsuperscript{35-36} Frequency distribution of grain counts over the megakaryocyte nuclei was determined and expressed as the ratio between the variance and the mean grain count \( \frac{\sigma^2}{\mu} \) according to Koburg.\textsuperscript{30}

Megakaryocyte Number

The number of megakaryocytes was counted in the histological sections of the femoral bone marrow fixed in Zenker-formalin solution and stained with either Giemsa tissue stain or hematoxylin and eosin. Only representative sections were used. They included 20 femora from each group of rats and 40 consecutive high-power fields were counted for each femur. The megakaryocyte number was expressed as average number per HPF (magnification \( \times 450 \)).

The student’s t test was adopted for statistical analysis of the data throughout this study.

RESULTS

Morphological Observations

Immune Thrombocytopenia. Many megakaryocytes in the rats of this group displayed a profoundly altered morphology. The most striking abnormalities were the hyaline appearance and the reduction of granularity in stage-III megakaryocytes. The latter feature was usually limited to the perinuclear portion of the cells. Cells with a finely granular cytoplasm, but without signs of platelet formation were also characteristically seen in stage-III megakaryocytes. Megakaryocytes of stage II also showed a distinct diminution of granularity in the perinuclear area. The cell nuclei often displayed bizarre forms with separation of lobes (Fig. 2). Megakaryocytes with excessive numbers of nuclei and increase in cell size were rarely seen.

In the thrombocytopenia group, the distinguishing morphological characteristic of the megakaryocytes was a very pronounced granularity of the cytoplasm and an increase in size of stage-III megakaryocytes. Furthermore, in
stage-II megakaryocytes, an early appearance of well-demarcated azurophilic granules in one large or in several discrete perinuclear areas of the cytoplasm was noted in almost every cell of this group. The majority of megakaryocytes of stage II and III had multilobulated nuclei (Fig. 3).

Megakaryocytes in the adsorbed antiserum group revealed no change from normal: megakaryocytes of stage I showed an intensely basophilic cytoplasm with no granularity; a clear lobulation of the nucleus was not observed in this stage. Megakaryocytes of stage II always had a faintly rose-colored cytoplasmic area with azurophilic granules. The cytoplasm in this stage was less intensely basophilic and had a foamy appearance. Clear lobulation of the nuclei was readily seen. Megakaryocytes of stage III showed an acidophilic cytoplasm with single granules or conglomerations of granules scattered throughout the cytoplasm.

**Differential Count of Megakaryocytes**

Differential counts of all three maturation stages of megakaryocytes in the immune thrombocytopenia group were significantly different from those in the thrombocytapheresis group and in the adsorbed antiserum group (Table 1). Counts of stage I and stage II were higher \((P < 0.01)\). Differential counts in the thrombocytapheresis group were similar to those in the adsorbed antiserum group. It should also be noted that no significant differences were found in megakaryocyte differential counts between rats of the same group sacrificed at different time intervals during the experiment.
Fig. 3.—Typical morphology of megakaryocytes in rats subjected to thrombocytopheresis. Stage-I megakaryocyte with nucleus showing multilobulation and heavy labeling. Large size of cell also observed (× 1000).

Labeling Index

The labeling index curves in the immune thrombocytopenia group clearly differed from those seen in the thrombocytopheresis and adsorbed antiserum groups (Fig. 4). The initial value of labeling index for stage-I megakaryocytes in the immune thrombocytopenia group (mean 23.5 ± 4.6 SD) was significantly lower ($P < 0.01$) than the initial value of labeling index for stage-I megakaryocytes in the thrombocytopheresis group (mean 47.7 ± 5.6 SD), and also lower ($P < 0.05$) than the initial value of labeling index in the adsorbed antiserum group (mean 33.0 ± 6.5).

Increase of labeling index of stage-I megakaryocytes and progression of the labeled cells through stages II and III were greatly delayed in the immune thrombocytopenia group, particularly when compared with the thrombocytopheresis group. This delay was best seen by observing at which time, in the various experimental groups of animals, the initially unlabeled stage-III megakaryocytes became labeled and the labeling index started to increase steadily. This increase was delayed in the immune thrombocytopenia group. Further-

Table 1.—One thousand megakaryocytes for each rat were studied. Values represent mean of 30 rats for each experimental group.

<table>
<thead>
<tr>
<th>Megakaryocyte Differential ± SD</th>
<th>Stage</th>
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<tr>
<td></td>
<td>I (%)</td>
</tr>
<tr>
<td>Immune Thrombocytopenia</td>
<td>24.0 ± 2.1</td>
</tr>
<tr>
<td>Thrombocytopheresis</td>
<td>18.8 ± 2.1</td>
</tr>
<tr>
<td>Adsorbed Antiserum</td>
<td>16.6 ± 1.4</td>
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more, the rate of increase of the labeling index of stage-III megakaryocytes between 24 and 36 hours was 1.25–1.50 per hour in the immune thrombocytopenia group. This value was significantly lower ($P < 0.01$) than in the thrombocytapheresis group (about 3.33 per hour), as well as in the adsorbed antiserum group (about 2.3 per hour). The different rates of increase in labeling index in the three experimental groups remained substantially unchanged throughout the subsequent hours and the peak values were reached between 54 and 60 hours for the immune thrombocytopenia group, at 48 hours for the platelet pheresis group, and between 48 and 54 hours for the adsorbed antiserum group. From all these data, it was concluded that the transit time through the different megakaryocyte maturation stages was significantly prolonged in the immune thrombocytopenia group, accelerated in the thrombocytapheresis group and normal in the adsorbed antiserum group.

It should also be noticed that the highest value in the labeling index curve of stage-III megakaryocytes in the immune thrombocytopenia group never reached the highest value of labeling index of stage II or I. This peak value, on the other hand, was reached in the thrombocytapheresis group and in the adsorbed antiserum group. Since this finding could reflect destruction of megakaryocytes as a consequence of the antibody action, grain counts of the labeled cells progressing from the DNA-synthesizing stage I to the DNA-non-
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synthesizing stage III were performed in an attempt to elucidate this problem.

Progression of Mean Grain Counts from Stage-I to Stage-III Megakaryocytes

In the immune thrombocytopenia group, a somewhat different pattern of progression of the mean grain count from stage-I to stage-III megakaryocytes was observed than in the other two groups (Fig. 5). At no time (except at the 18th hour), did the mean grain count of the initially nonlabeled stage III reach the mean grain count of the initially labeled stage-I megakaryocytes. The mean grain count of stage III at 18 hours was, however, derived from cells representing only about 1.1 per cent of the total population. The value of \( \frac{S^2}{m} \) was 1.2. This indicated that the fraction of cells was too small for a significant interpretation.\(^{36} \) Between 24 and 36 hours the mean grain count of stage-III megakaryocytes decreased in the immune thrombocytopenia group.

![Graph showing progression of mean grain counts from stage-I to stage-III megakaryocytes.](image)

**Fig. 5.**—Progression of mean grain counts from stage-I to stage-III megakaryocytes. Values of mean grain counts of stage-I megakaryocytes represented by solid circles on left of each panel; mean grain counts of stage-III megakaryocytes given by solid squares on right of each panel. Point represents mean value of three rats. Brackets represent ± 1 SD from mean (635–675 cells counted in three rats).
and continued to decrease during the following period of observation. During this period of time, the \( \frac{S^2}{m} \) was 5.6–5.7, indicating that the population of cells encountered was significantly large. At the same time, in the thrombocytopenia group, the mean grain count of stage-III megakaryocytes remained within the range of values seen in the megakaryocytes of stage I, except at 48 hours. It should be noticed that no labeled "naked" nuclei were observed in either group of animals at 24 to 36 hours.

**Number of Megakaryocytes**

The mean value of megakaryocyte number per HPF (Table 2) in the immune thrombocytopenia group was significantly lower \( (P < 0.01) \) than that obtained in the thrombocytopenia group. Both values, however, were significantly higher \( (P < 0.05) \) than in the adsorbed antiserum group.

**DISCUSSION**

Different patterns of megakaryocytopenesis emerged when sustained thrombocytopenia was experimentally induced by two different procedures: (1) injection of antiplatelet serum and (2) thrombocytopenia. These differences included: morphological appearance of the megakaryocytes, proportional distribution of the various maturation stages, rate of transit of the \(^{3}HTdR \) labeled cells through the serially connected cytological compartments, and megakaryocyte number.

Immaturity, lack of granulation in the cytoplasm, degenerative changes and other morphologic characteristics of the megakaryocytes, when thrombocytopenia was induced by means of platelet antibody, were also observed by other investigators both in experimental animals\(^{4,37,50} \) and in humans.\(^{2,3,34} \) Furthermore, similar megakaryocyte alterations were seen, particularly by Stefanini et al.,\(^{3} \) in idiopathic thrombocytopenic purpura (ITP), which we believe is an autoimmune disorder. On the contrary, when thrombocytopenia was experimentally produced in dogs by platelet pheresis, an increase in number of the megakaryocytes in the bone marrow with the appearance of early forms and lack of platelet budding were clearly observed, but severe degenerative changes with vacuolization both cytoplasmic and nuclear, and complete lack of granulation in the cytoplasm, as observed by us and by others\(^{4,37,50} \) with antibody, were not described.\(^{13} \) All these observations lead to the hypothesis\(^{38,8} \) that platelet antibody not only injures platelets in the circulation, but may also elicit an injurious effect on the megakaryocytes in the bone marrow, and that this combined effect is responsible for the thrombocytopenia. This hy-

<table>
<thead>
<tr>
<th>Megakaryocyte Number per HPF ± SD</th>
<th>Immune Thrombocytopenia</th>
<th>11.0 ± 0.25</th>
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<tr>
<td>Thrombocytapheresis</td>
<td>14.9 ± 0.67</td>
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<tr>
<td>Adsorbed Antiserum</td>
<td>9.1 ± 0.37</td>
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The present study demonstrates that in experimentally induced immune thrombocytopenia, the morphological alterations of the megakaryocytes and the changes in their proportional distribution into the various maturation stages ("shift to the left") cannot be regarded as an orderly compensatory mechanism by which the megakaryocyte renewal system responds to a sustained platelet demand in the peripheral circulation. In fact, under the conditions of our experiments, changes in compartment size of the various stages of megakaryocyte maturation were found to be due to impaired cell maturation.

If one makes the assumption that during the experiment, the overall DNA synthesizing time remained unchanged in the DNA synthesizing population of megakaryocytes, the initially low labeling index and its "sluggish" rise thereafter in stage-I megakaryocytes could be ascribed to one of three possible causes: a prolonged maturation time as a result of the deleterious effect of the platelet antibody on intracellular maturation of the megakaryocyte precursors, the failure of such megakaryocytes to incorporate 3HTdR, or the result of an increased influx of unlabeled cells from a precursor compartment maturing in parallel with the labeled cells. The second possibility can be ruled out by observing the time at which initially unlabeled megakaryocytes of stage III became labeled and proceeded in their steady increase. Had only the impaired incorporation of 3HTdR been responsible for the low labeling index of stage-I megakaryocytes, the transit time to the stages II and III would have been normal or increased in the case of normal or accelerated maturation. This was not observed. The third possibility cannot be completely discarded. An increasing influx of unlabeled cells into the labeled population due to large variations in the generative cycle of the unrecognizable megakaryocyte precursors could lead to underestimation of the actual maturation time. However, in the thrombocytopenia animals, the compensatory response of megakaryocytopenia to the sustained platelet depletion displayed a different pattern. An initially higher-than-normal labeling index of stage-I megakaryocytes and an accelerated transit time through the subsequent maturation stages in the presence of a normal proportional distribution of these stages, indicated an
orderly and well-balanced compensatory response to the low platelet count. These findings and the presence of an increased number of macromegakaryocytes in the thrombocytopenia group are consistent with the existence of compensatory megakaryocytopoiesis, as recently observed by Ebbe et al.\textsuperscript{48} in rats during recovery from acute thrombocytopenia. It appears, therefore, that the grossly altered kinetic pattern in the immune thrombocytopenia group reflected impaired and depressed maturation of the megakaryocytes as a consequence of the injury produced in these cells by the platelet antibody. At what level and to what extent the megakaryocyte renewal system was affected and the nature of the injury cannot be derived from this study. One might propose that the foreign serum itself, interfered with cellular enzymes necessary for megakaryocyte maturation. This possibility was excluded by the negative results in the control group injected with platelet-adsorbed foreign antiserum.

It is difficult to state with certainty from the kinetic data presented here that the antibody also caused destruction of some megakaryocytes during their maturation process, because data derived from grain counts do not have strictly quantitative value.\textsuperscript{44,46} Furthermore, the difference in mean grain counts between stage-I megakaryocytes initially, and stage-III megakaryocytes at 24 and 36 hours, was not large enough to support such an hypothesis. However, results derived from the megakaryocyte counts in the bone marrow sections indicated, indeed, megakaryocyte destruction. Although relative, these values showed that the mean megakaryocyte number in immune thrombocytopenia was significantly lower than after thrombocytopenia and this could be considered as an indication that some megakaryocytes had disappeared during maturation. It was of interest to observe that the mean megakaryocyte number in the bone marrow of rats with immune thrombocytopenia was higher than in the control rats which received adsorbed antiserum. This finding can be best interpreted as evidence that in the rats with immunologically induced thrombocytopenia the compensatory mechanism of megakaryocytopoiesis was not completely abolished by the antibody. More quantitative data will be needed to evaluate the extent of the antibody action on compensatory megakaryocytopoiesis. However, it appears from this study that the failure of megakaryocytopoiesis to establish a "new steady state" could be ascribed to the presence of ineffective megakaryocytopoiesis, presumably caused by the antibody action.

A "new steady state" indicating effective megakaryocytopoiesis was, on the contrary, achieved in the thrombocytopenia group. This finding demonstrates that in the rat, megakaryocytes are able to respond to, and to compensate for, an increased platelet demand. However, the exact mechanism by which this is accomplished is still not known. It has been suggested by Harker\textsuperscript{47} that the increase in total megakaryocyte number and the increase in their cytoplasmic volume are representative signs of the compensatory phenomenon. This suggestion is based on the results obtained after four and 10 days of sustained platelet depletion by pheresis in experimental animals. Since control of population size in a self-renewing system is dependent on the rate of "feed" into the transit population, and on the transit time of the
population units, the disturbance in megakaryocyte differential count reported after 10 days of thrombocytopenia by Harker would imply that effective megakaryocytopenesis could be achieved without establishing a “new steady state.” Although total megakaryocyte quantitation was not performed by us, it appears from our study that after six days of sustained platelet depletion a “new steady state” was indeed achieved by accelerating the rate of transit through an enlarged population of recognizable megakaryocytes.

It can be questioned whether these experimental conditions are appropriate enough to permit comparison with pathologic states in human beings. However, there is already evidence indicating that in humans with idiopathic thrombocytopenic purpura (an autoimmune disorder), or with thrombocytopenia produced by the infusion of platelet antibody, the compensatory response of the bone-marrow megakaryocytes to the low platelet count may be inadequate. This is demonstrated both by the morphological abnormalities and the changes in proportional distribution of the various megakaryocyte maturation stages; these are too striking in some cases of idiopathic thrombocytopenic purpura to be disregarded. Furthermore, the direct correlation at all degrees of thrombocytopenia found in human studies between reduction in platelet number and shortening of platelet survival would imply abnormality or absence of a compensatory increase of platelet production. On the other hand, post-hemorrhagic thrombocytosis in which replacement was performed with platelet-poor blood provides evidence that bone-marrow megakaryocytes possess the ability to respond promptly to an increased body's demand for platelets.

Thus, increasing experimental and clinical evidence supports the hypothesis that in human disease with immunologic thrombocytopenia, the bone marrow fails to react properly to the stimulus of a low platelet count and that this can be ascribed to the injurious effect of platelet antibody on megakaryocytopenesis and, therefore, on platelet production. Consequently, the very low platelet count of many cases of “ITP” (autoimmune thrombocytopenia) may be due to a double disturbance, i.e., reduced platelet production by the megakaryocytes and increased platelet destruction.

**Summary**

The hypothesis that in immune thrombocytopenia, platelet antibody may not only cause destruction of the circulating platelets but also depress platelet production by injuring the megakaryocytes of the bone marrow, was tested experimentally.

Sustained thrombocytopenia was produced in rats by titrated injections of a potent heteroimmune antiplatelet serum and megakaryocytopenesis was then studied by the use of tritiated thymidine and bone marrow autoradiography. Rats in which the platelet count was maintained at a lower than normal level by repeated thrombocytopenies, and other rats injected with platelet antiseraum previously absorbed with rat platelets, served as controls.

Profoundly altered patterns of megakaryocytopenesis were found in the rats in which thrombocytopenia was produced by the antiplatelet serum. The data indicated a severely impaired and depressed megakaryocyte maturation and,
possibly, destruction of some of the megakaryocytes during their maturation process. In the rats in which the platelet level was maintained low by repeated thrombocytophereses, the pattern of megakaryocytopoiesis indicated accelerated maturation and there was also an increased megakaryocyte mass. No difference from normal was found in the rats receiving the platelet-adSORbed antiserum. It was concluded that the platelet antibody produced an injurious effect on the megakaryocytes in the bone marrow, thereby depressing platelet production, and that the immune thrombocytopenia was the result of both increased platelet destruction and defective platelet production.

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