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Platelet Recovery after Induction of Acute Thrombocytopenia[1] (38642)

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An experimental model of thrombocytopenia in animals produced by injection of antiplatelet serum (APS) has been used to study thrombocytopenia (1–8). In addition, animals in rebound thrombocytosis after induction of thrombocytopenia with APS have been used in assays of thrombopoietin (9, 10). However, the response of platelets to various degrees of thrombocytopenia induced with APS has not been systematically investigated. The experiments reported here compare platelet recovery among groups of rats injected with different amounts of antisera. The results provide new information about dose effects of APS in producing thrombocytopenia, and about platelet recovery. The results on time sequences and magnitudes of platelet response provide a background for effective experimental use of animals with induced thrombocytopenia.

Materials and Methods. Sprague–Dawley derived rats with an average weight of 396 g were injected with different volumes of antiplatelet serum intravenously (saphenous vein) or intraperitoneally, and platelet counts were taken at intervals. Platelet counts were made with the phase microscope method on blood taken from the saphenous vein. To reduce variability due to differences among rats in normal platelet counts, serial platelet counts of individual rats were translated to percentages of the pretreatment platelet count of the same rat. The average pretreatment count was $0.962 \times 10^6/mm^3$ (55 rats).

Antisera to rat platelets were made in rabbits as previously described (4). The volumes of antiserum injected ranged between 0.02 and 2.0 ml. Since preliminary experiments had shown that the low point of the platelet count after injection of APS was often reached at 6 or 7 hr, the first count was usually taken at 6 hr rather than sooner after injection of APS.

Results. Circulating platelet counts were reduced to different levels and for varying lengths of time by varying the volume and number of injections of APS (Table I). Volumes of 0.02–0.10 ml usually produced mild to moderate thrombocytopenia (platelet count at 6 hr > 10% of pretreatment count), while larger volumes (0.2–2.0 ml) produced more severe and longer-lasting thrombocytopenia. Repeated doses once a day for either 2 or 4 days maintained a low platelet count during dosage. There was some variation among rats in the degree of thrombocytopenia produced by injection of a given volume of antiserum. However, the return pattern of the platelet counts among rats that experienced a similar initial depth or duration of thrombocytopenia was similar (Fig. 1). Untreated rabbit serum did not lower the platelet count (Group A).

The course of recovery of the circulating platelet mass was variable, depending on the degree and duration of depression. When the platelet count at 6 hr after injection of antiserum was 66% of the pretreatment count (Group B), the peripheral platelet count appeared to increase at a fairly constant and moderate rate (about 14%/day) for 4 days (Fig. 2), reaching its peak at 5 days. When the count was depressed to 40% at 6 hr (Group C), the rise was moderate for about 36 hr, perhaps not different from Group B, and then became more rapid sometime between 36 and 48 hr. Likewise, when the count was reduced to essentially zero by platelet antiserum, there was a period of moderate rise followed by a much more rapid rate (Group E). It appeared that the second rate of increase became greater as the degree and duration of depression were greater (Groups C-G). For example, the increase in group D, whose average platelet count was 15% at 6 hr, was about 300,000 platelets/mm^3 of blood per day between 48 and 96 hr, while in

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TABLE 1. DEPRESSION AND RETURN OF PLATELETS AFTER INDUCTION OF THROMBOCYTOPENIA.

Group	Platelet Count Depression		Linear regressions of platelet counts on time after APS								Population Increase (platelets/mm³ per day × 10^{-3})	Injection	
	hr[a]	%[b]	Y Intercept[c]	Slope (%/hr)	SE of slope	Time[d] (hr)	SD[e]	Correlation Coefficient	Observations per rat	Number of rats		No.	Route
A	6	104	102	0.07	0.022	3–96	2.4	0.68	13	11	17	1	iv
B	6	66	61	0.58	0.053	3–96	5.2	0.97	5–10	3	139	1	iv
C	6	40	38	0.42	0.115	3–36	3.4	0.88	2–6	6	101	1	iv
			25	0.80	0.092	48–96	3.7	0.97	6	6	192		
D	6	15		1.30	0.076	48–96	3.0	0.99	6	3	312	1	iv
E	24	12	−4	0.63	0.151	12–36	2.7	0.95	4	3	151	1	iv
			−44	1.67	0.113	48–102	5.8	0.99	8	3	404		
F	36	11	−16	0.75	0.048	24–36	0.4	~1.00	3	4	180	1	iv
			−62	1.94	0.098	48–96	4.4	0.99	10	4	466		
G	54	14	−161	2.87	0.200	72–120	7.7	0.99	5	2	689	2	ip
H	72	10	−170	2.44	0.103	72–144	5.5	~1.00	4	6	586	1	ip
I	96	15	−226	2.33	0.112	109–168	5.6	~1.00	6	2	559	4	ip
J	120	12	−322	2.79	0.096	120–168	3.3	~1.00	3	2	670	1	ip

[a] Time in hours after injection of APS.

[b] Percent of pretreatment platelet count (observed). The count at 6 hr is given where this count was greater than 10%; otherwise a later count between 10 and 15% during the initial period of recovery is given along with the time when the count was taken.

[c] Percent of pretreatment platelet count.

[d] Period for which regression was calculated.

[e] Standard deviation of the deviations from regression.

group F, whose average count was 11% at 36 hr, it was 466,000, and in group J, whose platelet count remained depressed for about 5 days, it was 670,000. When platelet counts began to increase within a day after injection of APS, the break between a moderate and a more rapid rate of increase occurred around 36–40 hr after injection.

The average platelet count of 11 rats injected with normal rabbit serum varied between +10 and −2% of the average pretreatment platelet count during a 5-day period (Group A). A regression calculated for the 3–96 hr period had a very small positive slope (0.07%/hr) and a low correlation coefficient in comparison with the APS groups.

In some of these experiments it was possible to estimate the peak elevation of the platelet counts and the approximate time when the peak was reached. When an essentially zero platelet count persisted for up to a day after injection of APS, the time of maximum count occurred around 125–130 hr after injection. When minimal platelet count was prolonged for more than a day, the peak time was likewise postponed. The maximum count increased in relation to the severity of platelet depression. The maximum platelet count rarely exceeded twice the pretreatment count and was frequently around 180–190%

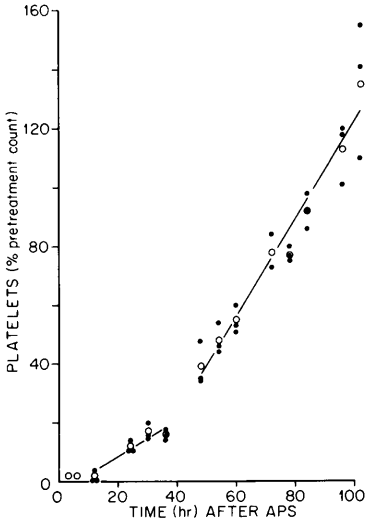

FIG. 1. Platelet counts (percent of pretreatment count) of rats after intravenous injection of antiplatelet serum (Group E, Table I). Solid circles represent counts of individuals, open circles are the averages, and lines are linear regressions of average counts on time after APS.

after severe thrombocytopenia, less after moderate thrombocytopenia. It was also noted that the length of time between the beginning of the "second phase" of platelet increase and attainment of the maximum count was about 80–90 hr.

Discussion. These experiments have dem-

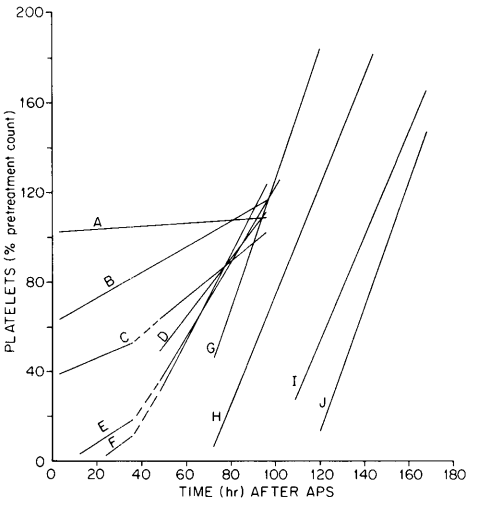

FIG. 2. Linear regressions (solid lines) of average platelet counts (percent of pretreatment count) on time after APS in groups of rats injected with different amounts of APS (Table I).

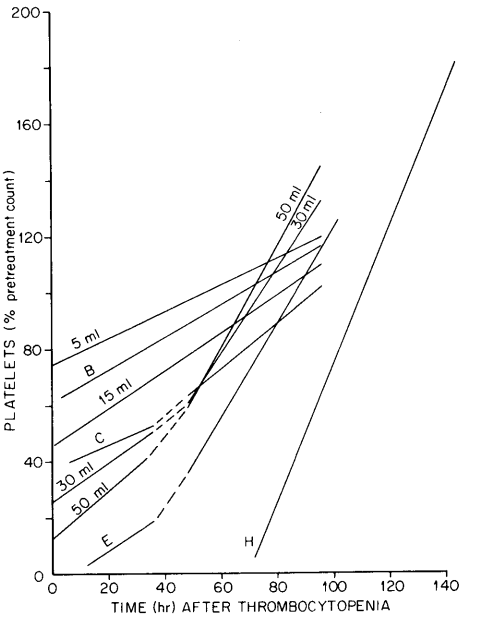

FIG. 3. Linear regressions of average platelet counts (percent of pretreatment count) of Groups B, C, E, and H of Table I, and of groups of rats after exchange transfusion with 5, 15, 30, or 50 ml of platelet-poor blood. The methods by which platelet-poor blood was exchanged are described in Ref. 11.

onstrated that platelet recovery after acute thrombocytopenia induced with APS differs, depending upon the degree and duration of the thrombocytopenia induced.

The general patterns of platelet recovery in antiserum-induced thrombocytopenic rats were similar to those observed in rats made thrombocytopenic by exchange transfusion with different amounts of platelet-poor blood (11) (Fig. 3). After mild thrombocytopenia, the peripheral platelet count increased at a moderate rate for 4 days in both cases (Group B, and 5 and 15 ml). After moderate to severe thrombocytopenia, induced either by platelet-specific antiserum (Groups C and E) or by exchange transfusion with platelet-poor blood (30 and 50 ml), an early moderate rate of platelet increase was followed by a second phase of more rapid platelet increase.

The moderate platelet increase may result in part from changes in the age composition of the circulating platelet population (11). Assuming that platelet survival is determined partly by platelet age, average platelet age in individuals recovering from thrombocytopenia will be younger than normal, and therefore the rate of exit from the population may be reduced. The moderate rate of platelet increase may also result in part from release of platelets from a small reserve pool. Comparisons of models of recovering platelet populations with observed responses to thrombocytopenia indicate, however, that these factors of age composition of the population and a reserve pool do not fully account for the sustained platelet increase over a 5-day period, including the overshoot above normal platelet levels. The results therefore suggest that some new production of platelets takes part in the recovery and overshoot observed in rats responding to moderate thrombocytopenia. Indeed, studies of labeled thymidine uptake by megakaryocytes have suggested that changes in megakaryocytopoiesis contribute to added platelet production after moderate thrombocytopenia (12).

The rapid platelet increase in the second phase is sustained by several changes in megakaryocytopoiesis. In thrombocytopenic rats there is an increase in average ploidy level of megakaryocytes, in the size of megakaryocytes, in the size of the megakaryocyte population, and in the rate of maturation of megakaryocytes (2, 6, 13). The development of these changes in megakaryocytopoiesis requires a finite period of time. Observations

of megakaryocytopoiesis in thrombocytopenic rats suggest that the ploidy increase (6) and the introduction of new megakaryocytes from a precursor pool (14) make their major contributions to new platelet production between 48 and 96 hr after induction of acute thrombocytopenia, the same time period during which the rapid second phase of platelet increase was seen (when platelet count had been markedly reduced).

Various mechanisms can be suggested to explain the prolonged thrombocytopenia after relatively large doses of APS including (i) a persistance of circulating platelet antibodies (15), and (ii) direct damage to megakaryocytes by platelet antibodies (1, 7, 15, 16). It is clear from immunofluorescence studies that antibodies against platelets can also attach to megakaryocytes (17). The platelet production results presented in this paper suggest, however, that relatively small doses of APS produce little or no damage to megakaryocytes, because platelet production continues within a few hours after injection. Moreover, platelet return after mild to moderate thrombocytopenia was similar whether thrombocytopenia was induced by APS or by exchange transfusion with platelet-poor blood (Fig. 3). However, the prolonged platelet depression after larger doses may indeed result from damage to megakaryocytes by the introduced antibodies. In assessing megakaryocyte changes after APS it is important to distinguish between possible effects of injected platelet antibodies on megakaryocytes, and megakaryocyte changes that occur in response to thrombocytopenia.

The growth rate of the platelet population in the second phase became greater as the severity and duration of thrombocytopenia increased (Fig. 2, Table I). However, the results suggest that the maximum rate of platelet increase may be elicited by about 2 days of almost complete absence of circulating platelets. Moreover, the maximum platelet count increased as the initial severity of thrombocytopenia increased until the peak count reached about 180–200% of pretreatment count, but more severe or prolonged thrombocytopenia apparently had no further effect on the maximum count. These findings indicate that the marrow has a maximum limit of platelet production. The rate of platelet population increase (not necessarily the same as platelet production rate) in rats exhibiting this maximum response is approximately three times the rate of platelet production in untreated rats, when the latter estimate is based on an average platelet life span of 4.5 days (18).

The variable platelet production in relation to degree and duration of thrombocytopenia raises questions about mechanisms responsible for the graded responses. Are variable numbers of cells stimulated to differentiate from megakaryocyte precursors? Are variable numbers of maturing megakaryocytes stimulated to undergo an additional replication of DNA? Is a moderate response supported primarily by one mechanism and a marked response by another? The commonality of time periods during platelet response (e.g., similar time of maximum platelet count, except after prolonged thrombocytopenia; similar 80–90 hr time period from second phase of rapid platelet increase to peak count) suggests that the general mechanisms for responding to induced, acute thrombocytopenia are similar regardless of the degree of stimulation, but that quantitative aspects vary.

The variable quantitative and qualitative responses seen in platelet production in relation to severtiy and duration of thrombocytopenia and the underlying mechanisms have important implications when APS is used to prepare animals in rebound thrombocytosis for use in assays of presumptive thrombopoietic substances. Differences in doses of APS can markedly affect the time and magnitude of response of animals and thereby their suitability as assay subjects. It will therefore be important in assay experiments using subjects in rebound thrombocytosis to determine platelet recovery responses in relation to dose of APS, and to make platelet counts on assay subjects at selected times during platelet recovery to determine the nature of that recovery.

Summary. After mild thrombocytopenia (about 50–100% of control platelet level), induced by injection of antiplatelet serum (APS), platelets increased at a fairly constant and moderate rate for about 4 days and reached a maximum count, greater than pretreatment levels, on the fifth day. After

moderate to severe thrombocytopenia, an early moderate rate of platelet increase was succeeded within 2 days by a second more rapid rate that persisted for about 3 days. The maximum overshoot of the platelet count was usually less than twice the pre-treatment level. After the largest doses of APS, the platelet count remained depressed for several days. Platelet response after acute thrombocytopenia induced with antiserum is therefore variable, depending on the level and duration of thrombocytopenia.

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