THE ADHESION OF DOG PLATELETS TO BACTERIA

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ALTHOUGH it is known that platelets of certain animals adhere to bacteria, red cells, and other foreign particles, the fundamental mechanism responsible for this reaction is poorly understood. For this reason, investigations have been carried out in this laboratory in an attempt to define more concisely those factors controlling or affecting the adhesiveness of platelets to foreign bodies. The findings of Govaerts,[1,2] Roskam,[3,4] Delrez,[5] and Aynaud[6] concerning the adhesion of rabbit platelets to bacteria have been verified and extended.[7] Govaerts[1] noted that, whereas rabbit platelets did not adhere to pneumococci, dog platelets were adhesive to this organism. The study of the adhesion of dog platelets, reported below, was made in an attempt to determine the nature of the species differences in the platelet-bacteria adhesion reaction.

PROCEDURES AND RESULTS

1. Adhesion of Dog Platelets to Bacteria in Rotating Systems

All blood samples were obtained by venipuncture from the jugular vein. Blood containing anticoagulant* was centrifuged at 1500 rpm for approximately 10 minutes in order to obtain platelet-rich plasma. Serum and defibrinated blood which had been clarified by centrifugation were added to suspensions of platelets isolated from citrated dog blood.[8] All plasma, sera and blood samples were kept chilled during the tests by immersion in cracked ice. The majority of these samples were used within 1 to 4 hours after blood withdrawal. One cc. amounts of formalized bacterial suspensions adjusted to the turbidity of a MacFarland nephelometer no. 3 were sedimented by centrifugation and resuspended in 0.4 cc. of 1:2 dilutions of platelet-rich bloods. These mixtures were rotated at 1 rpm for 15 minutes at 37° C., diluted 1:2 with brilliant cresyl blue dye C. I. no. 877 (0.5 per cent final concentration in 3.8 per cent sodium citrate), and examined microscopically for 5 minutes.

The degree of mixed clumping of bacteria and platelets was recorded as ++++ when mixed clumps larger than 2 oil-immersion fields were seen using 10 × oculars; +++, ½ to 2 fields; ++, 100 platelets to ½ fields; +, 25 to 100 platelets; ±, many small mixed aggregates; trace, several small aggregates.

Rotation tests were performed with platelet-rich oxalate plasma from 3 dogs; citrate plasma, serum, and defibrinated blood from 2 dogs. The results are presented in table 1. All strains of streptococci and staphylococci tested were observed ensnared in large clumps of platelets in the oxalate plasma, serum, and defibrinated blood. The degree of mixed clumping was recorded as less than 3+ in only 2 of 56

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*One part of 3.8 per cent sodium citrate, or 0.1 M sodium oxalate to 4 and 7 parts of blood, respectively.

tests. In contrast, mixed adhesion was much less marked in citrate plasma, being recorded as + or less in 9 of 24 tests.

To determine if platelets *per se* were adhesive, platelets were isolated from 2 dogs and suspended in saline to a concentration approximating that in the above tests. These suspensions were mixed with the 12 strains listed in table 1, and rotated. In 12 of the 24 tests no adhesion was observed; in the remaining 12 tests only a trace of adhesion was seen.

TABLE 1.—*Adhesion of Dog Platelets to Streptococci and Staphylococci in Rotated Dog Sera and Plasmas*

Bacteria tested	Degree* of Mixed Clumping of Platelets and Bacteria								
	Oxalate plasma			Citrate plasma		Serum		Defibrinated blood	
	Dog no.			Dog no.		Dog no.		Dog no.	
	B	C	D	C	G	G	A	A	D
S. pyogenes	++++	++++	++++	+++	+++	++++	+++	++++	++++
S. fecalis No. 1430	+++	+++	++++	+	+	++++		+++	
S. fecalis Ree	+++	+++	++++	+++	+++	+++	+++	+++	+++
S. salivarius St5E	++	++++	++++	++	tr.	+++	++	+++	+++
S. mitis	++++	++++	++++	+++	+++	+++		++++	++++
S. aureus	++++	++++	++++	++++	+	++++	+++	+++	
S. citreus	+++	++++	++++	+	+				
S. bovis	++++	++++	+++	+++	++				
S. salivarius No. 1431	++++	++++	+++	+	++				
S. fecalis Bay	++++	++++	++++	++++	+++				
S. equinus	++++	+++	++++	++	±				
S. fecalis No. 1435	++++	+	++++	+++	+				

* ++++ = marked mixed clumping; ± and tr(ace) = very weak mixed clumping. See text for details.

2. *Titration of the Adhesive Factor*

Sedimented suspensions of isolated platelets and *S. fecalis* no. 1430 were resuspended in serial dilutions of oxalate and citrate plasma, serum, and defibrinated blood. It can be seen from table 2 that activity of the factor inducing platelet adhesion was still apparent in oxalate plasma, serum, and defibrinated blood which had been diluted to 1:64 and, in some cases, to 1:128. The adhesiveness of platelets to *S. fecalis* no. 1430 was depressed when citrate plasma was employed. It is interesting to note that, although the adhesion reaction was generally depressed in citrate plasma, there were significant differences in adhesiveness to the various bacterial species. In addition, the evidence indicates that citrate plasmas from different dogs may exhibit varying degrees of adhesiveness. This selectivity was, in general, not apparent in oxalate plasmas, sera, or defibrinated bloods.

3. *The Nature of the Adhesive Factor*

Antibodies have been implicated in the Rieckenberg phenomenon,[9] in the formation of platelet-thrombi in bacterial endocarditis,[10] and also in the platelet-bacteria adhesion reaction.[1] Therefore, it was deemed advisable to investigate the role of bacterial agglutinins in dog plasma in the platelet-bacteria adhesion reaction.

The titers of the agglutinins in 4 dog oxalate plasmas for the 12 strains used throughout this study were found to be extremely variable; however, the differences in titers of the 4 plasmas for the same strains were not marked. Agglutinin titers of 1:64 or higher occurred in only 9 or 48 tests, whereas titers of 1:8 or less occurred 29 times. Since the platelets in oxalate plasmas adhered to all bacteria tested, irrespective of the presence or absence of bacterial agglutinins, it appears that agglutinins are not the responsible adhesive factor, a conclusion also reached

TABLE 2.—*The Potency of the Adhesive Factor in Dog Plasma and Serum*

Dog number	Type of Blood	Degree of Mixed Clumping of Platelets and Bacteria					
		Dilutions of Blood					
		0	½	⅛	1/32	1/64	1/128
C	Oxalate plasma	+++	+++	++	+	±	tr.
E	" "	+++	+++	++	±	±	tr.
C	Citrate "	±	±	tr.	tr.	o	o
G	" "	±	+	tr.	tr.	o	•
G*	" "	±	++	++	tr.	o	o
D	Serum	+++	+++	+	+	±	tr.
G	Defibrinated blood	++++	+++	++	±	±	o

* *S. fecalis* Bay used in this test. *S. fecalis* No. 1430 used in all other tests.

from investigations of this reaction in rabbit blood.[7, 11] This assumption was further verified by the finding that the agent in dog blood, as in rabbit blood,[1] which presumably induces adhesiveness is inactivated by heating at 56° C. for 30 minutes (table 3).

The susceptibility to shaking of the factor causing adhesion was also tested. Serum was shaken 270 times per minute in a stoppered tube for 15 hours at room temperature. Isolated platelets were then added to the serum, and to unshaken serum, and rotated with *S. pyogenes*, *S. aureus*, and *S. fecalis* no. 1430. From table 3 it is seen that the adhesive factor is partially inactivated by shaking.

The ability of bacteria to adsorb the adhesion-inducing factor from serum was then investigated. Two cc. of a 1:2 dilution of sera previously found to contain demonstrable agglutinins were added to 0.1 cc. of the packed, specific bacterial cells. Agglutinins were adsorbed from serum until they were exhausted or could be removed by low dilution. Isolated platelets were added to adsorbed and unadsorbed sera and rotated with specific and nonspecific bacteria. The data presented in table 3 indicate that the bacteria had effected the removal of the adhesive factor for both the specific and nonspecific bacteria. Only the platelets in the sera of Dog A adsorbed with *S. salivarius* St5E still possessed any adhesive ability, and that was very weak.

Sera from 2 dogs were also added to Kieselguhr, using 1 part of washed, packed Kieselguhr to 4 parts of serum. Adsorption was carried out twice for 30 minutes at 37° C., and the sera then clarified by centrifugation. Isolated platelets were added to adsorbed and unadsorbed sera, and the mixtures rotated with *S. pyogenes*, *S. aureus*, and *S. fecalis* no. 1430. The platelets in the adsorbed sera exhibited no tendency to adhere to the bacteria (table 3), indicating complete removal of the adhesive factor.

To determine whether or not soluble bacterial substances might induce some clumping of platelets, the supernatant fluids of all sedimented formalized bacterial

TABLE 3—*Inactivation of the Adhesive Factor in Dog Serum by Heating, Adsorption, and Shaking*

Dog number	Treatment of Sera	Degree of Mixed Adhesion of Platelets and Bacteria									
		S. salivarius St5E		*S. pyogenes* No. 1408		*S. aureus* No. 1307		*S. bovis* No. 1425		*S. fecalis* No. 1430	
BG	None	++++		++++		++++		++++		+++	
	Heated*	0		0		0		0		0	
AD	None			++++	++++	++++	++++			++++	++++
	Shaken			+	++	++	++			++	+++
AD	None			++++	++++	++++	++++			++++	++++
	Adsorbed with Kieselguhr			tr.	tr.	0	0			0	0
AD	None	+++	+++	++++	++++	+++	++++	++++	++++	+++	++
	Ads. with *S. salivarius* St5E	tr.	tr.	±	tr.			±	±		
	Ads. with *S. fecalis* number 1430			tr.	tr.			0	0	0	0
	Ads. with *S aureus* number 1307			tr.	0	tr.	0	0	0		

* Heated to 56° C. for 30 minutes.

suspensions were rotated in citrate platelet-rich plasma. In no instance did these fluids cause clumping of the platelets.

DISCUSSION

The results obtained indicate that the factor in the plasma resonsible for the adhesion of dog platelets to bacteria is complex, and that the identification of this factor will undoubtedly require considerable chemical and immunologic study. This factor in the plasma is tentatively designated as the PAD factor for convenience, and also to distinguish it from the possibly unrelated factor responsible for the agglutination of platelets.[12]

Certain evidence has been adduced, however, which permits the exclusion from consideration of some of the known plasma factors. The possible identity of the platelet-bacteria adhesive factor with prothrombin is excluded by the demonstration that the factor is present in serum. Likewise fibrin, considered by some investigators to be the cause of platelet "stickiness," is eliminated from consideration because of the undiminished potency of the adhesive factor in defibrinated blood and serum.

It appears that the bacterial agglutinins in dog blood, as in rabbit blood,[7]

are not responsible for the adhesion of platelets to bacteria. It would be technically difficult to obtain direct evidence of this point by specific adsorption and elution of the agglutinins since the adhesive factor is inactivated in the process of adsorption. The following properties of the PBA factor differentiate it from bacterial agglutinins: (a) thermolability; (b) adsorption by nonspecific agents; (c) a high and fairly constant titer in sera of all dogs.

The possibility that the complement complex and opsonin are related to the PAD factor was considered in order to design future experiments; however, any such suggestion of relationship at the present time is conjectural. The PAD factor is more stable than one might expect dog hemolytic complement to be since it is still active, although decreased in activity, after storage for one week at 8–10° C., and is only partially inactivated by shaking at room temperature for 15 hours. In addition, the activity of the PAD factor is depressed by sodium citrate, confirming earlier studies[4]; whereas it is reported that sodium citrate does not depress complement activity.[8]

The platelet-bacteria adhesion reaction is marked in both rabbit and dog bloods. In these animals there is definite evidence of specificity in the action of platelets in *citrate* plasma upon the various bacterial species. However, there are some differences in the action of the platelets upon certain bacteria. For example, rabbit platelets adhered intensely to all strains of *S. fecalis* tested, whereas dog platelets adhere very weakly to fecalis organisms in some instances. There were also differences in the adhesion of dog and rabbit platelets to *S. salivarius*. Whether these varying results reflect quantitative or qualitative differences in the PAD factors in dog and rabbit blood is not known. In order to develop this thought more fully, it would be necessary to explain why varying degrees of adhesion are noted in citrate plasma but not in dog oxalate plasma, serum, and defibrinated blood. However, it should not be construed, in view of our lack of knowledge of this subject, that this factor is responsible for the general "stickiness" of platelets under all conditions. The relationship of the platelet *agglutinating* factor, described by Copley and the author,[12] to the platelet-bacteria adhesive factor will be discussed in subsequent reports.[13, 14]

While the nature of the adhesion reaction is not known, certain opinions are invited. For example, Govaerts has stated that a plasma factor "opsonizes" the cell surfaces, presumably, as a foreign particle is prepared for phagocytosis by white blood cells. However, the platelet adhesion reaction apparently differs from the process of phagocytosis in two ways: (1) it does not appear to be enhanced by or concerned with antibodies; (2) the introduction of the foreign particle into the blood causes a rapid, complete disruption of the platelet stability, so that the platelets adhere firmly, not only to the particle, but to other platelets. The sudden disruption of the stability of the platelets following the introduction of bacteria into the blood does resemble in certain respects the rapid elimination of platelets from the circulating blood during anaphylactic shock.

SUMMARY

Streptococci and staphylococci, when added to platelet-rich plasma, serum, and defibrinated blood of the dog, are quickly enmeshed by large clumps of platelets.

The factor responsible for this reaction is present in the plasma. It is heat-labile, is inactivated by adsorption with Kieselguhr and bacteria, and is partially inactivated by shaking. The activity of this factor is depressed in the presence of sodium citrate.

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