Platelet Function in Leprosy [1]

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In the past few years, defective platelet function has been recognized in a number of dysproteinemic states such as multiple myeloma, Waldenstrom's macroglobulinemia and rheumatoid arthritis. Impairment of platelet adhesiveness ([4, 14, 15]) aggregation to ADP, thrombin and collagen ([6, 18, 19]) and reduced availability of platelet factor 3 ([13]) have all been considered to result from accumulation of abnormal proteins in the blood in these disorders. Leprosy is a classical example of a disease in which multiple protein abnormalities are known to occur ([2, 10, 11]). It is also a general observation that thromboembolic phenomena are less frequent in leprosy ([17]). Whether this has any relation to altered platelet function brought about by protein abnormalities in this disease is not known. The present study reports tests of platelet function in relation to immunoglobulins performed on 50 patients having either tuberculoid or lepromatous leprosy.

MATERIALS AND METHODS

Fifty patients, 35 of whom suffered from lepromatous and 15 from tuberculoid leprosy for periods varying from 6 months to 20 years, were investigated. None of the patients gave a history or showed any clinical evidence of bleeding, nor had they received aspirin or any other drugs known to alter platelet function in the preceding ten days. The diagnosis in each instance was based on clinical presentation, demonstration of acid-fast bacilli in skin scrapings, or skin and lymph node biopsies as recommended by Ridley and Jopling ([16]). Tests of platelet function and routine estimation of hemoglobin, packed cell volume and platelet counts were performed on all patients while immunoglobulins were determined on 30 of them.

Hemoglobin as cyanmethemoglobin and packed cell volume were determined according to the procedure described by Dacie ([7]). Platelet counts were performed using ammonium oxalate as a diluent ([9]). Bleeding time was recorded by Ivy's method as described by Dacie ([7]), and clot retraction was determined by the method of Biggs and Macfarlane ([1]).

Platelet adhesiveness *in vivo* was tested by the method of Borchgrevinck ([3]). Platelet aggregation to ADP was measured as described by Hardisty and Ingram ([9]), and to collagen according to the method of Zucker and Borelli ([20]). In ten of the patients, platelet aggregation to collagen was also measured after its treatment with plasma from patients having leprosy. For this, 0.2 ml of freshly prepared collagen suspension was incubated with an equal volume of platelet poor plasma from the patient. Each test was run with a control employing collagen incubated with normal platelet poor plasma. Platelet factor 3 availability (Pf-3a) was determined by the method based on kaolin clotting time of dilutions of platelet rich plasma as described by Hardisty and Hutton ([8]). The estimation of the three major human immunoglobulin fractions IgG, IgM and IgA was carried out by the method of radial immunodiffusion in plates according to the technic of Mancini *et al* ([12]).

RESULTS

A high frequency of platelet defects was observed in leprosy. In as many as 44 of the 50 patients some platelet abnormality was detectable. Of note were the abnormalities in platelet adhesiveness and their aggregation to collagen that were impaired respectively in 27 and 23 of the 50 patients (Table 1). Their frequency, however, differed in the lepromatous and tuberculoid types. While the platelet adhesiveness was impaired in 47% of the tuberculoid patients, 55.5% of the lepromatous patients were so affected. Similarly, defective aggregation of platelets to collagen was a feature in 54% of lepromatous but only in 27% of tuberculoid patients. ADP induced aggregation was

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TABLE 1. *The frequency of platelet function defects in the polar types of leprosy.*

Platelet defect	Lepromatous leprosy[a] (35)		Tuberculoid leprosy[a] (15)	
	No. patients	%	No. patients	%
Thrombocytopenia	4	11.4	1	6.6
Prolonged bleeding time	3	8.5	1	6.6
Poor clot retraction	1	3.0	0	0.0
Reduced Pf-3a	10	28.5	4	26.6
Reduced platelet adhesiveness	20	55.5	7	46.6
Reduced platelet aggregation to:				
ADP	3	8.5	3	20.0
Collagen	19	54.3	4	26.6

[a]Figures in parentheses represent total number of patients in each group.

TABLE 2. *Severity of defective platelet function in 50 leprosy patients.*

Test	Lepromatous leprosy[a] (35)	Tuberculoid leprosy[a] (15)	Control[a] (10)
Platelet adhesiveness[b]	24.6 ± 13.1 $p < 0.005$ $t = 2.65$	28.3 ± 4.2 $p < 0.05$ $t = 1.5$	36.0 ± 6.5
Platelet aggregation to:			
Collagen[b]	27.4 ± 11.5 $p < 0.0005$ $t = 3.93$	36.4 ± 20.1 $p < 0.20$ $t = 0.78$	40.8 ± 10.8
ADP[c]	10.0 ± 2.4	11.0 ± 2.7	9.3 ± 1.8
Pf-3a index[b]	3.4 ± 2.7	8.4 ± 2.1	> 25

[a]Figures in parentheses represent number of patients in each group.
[b]Results given as percent ± S.D.
[c]Results given as seconds ± S.D.

not so common a defect. In one fourth of the patients of either group, availability of Pf-3 was inadequate. Prolongation of bleeding time and thrombocytopenia were infrequent.

The changes in platelet adhesiveness, aggregation and availability of Pf-3 are compared with the normal in Table 2. Reduction in platelet adhesiveness, although observed in both groups of patients, was statistically more significant in lepromatous as compared to tuberculoid patients. Similarly, impairment of platelet aggregation to collagen was much more significant in lepromatous patients. Although the frequency of defective availability of Pf-3 was similar in the two groups, its decrease was more profound in the lepromatous patients. Platelet count, bleeding time and clot retraction were not significantly different from the normals subjects.

Figure 1 shows the severity of each of the major defects of platelet function. It is clear that among the patients who showed these defects, in more than 50% the platelet adhesiveness was below the minimum normal of 25% and aggregation to collagen was below this level in 46% of the patients.

The immunoglobulin levels in patients of

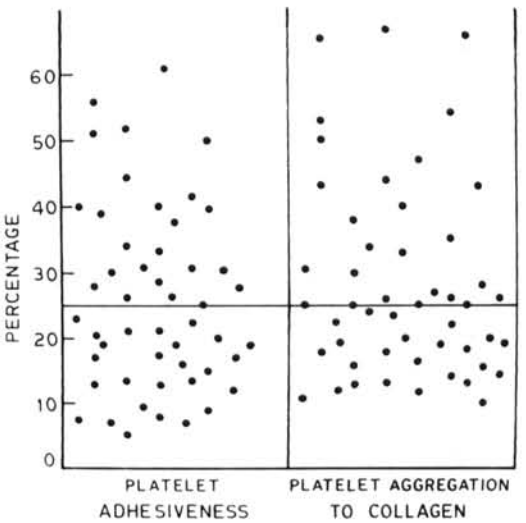

FIG. 1. Platelet adhesiveness and platelet aggregation to collagen in 50 patients having leprosy. The minimum normal for each is 25%.

leprosy are summarized in Table 3. While the pattern of distribution of different immunoglobulins in these patients was the same as in the normal subjects, important quantitative differences were observed with respect to IgM. On an average, the IgM values were 253 and 294 mg% in lepromatous and tuberculoid leprosy respectively, as compared to the normal mean of 155 mg%. These differences were statistically highly significant ($t = 4.86$, $p < 0.0005$ in lepromatous; and $t = 8.77$, $p < 0.0005$ in tuberculoid). The apparent increase in IgA and decrease in IgG was without any statistical significance in either of the groups.

Since the increase in IgM was the most remarkable change in the immunoglobulin composition of these patients and alterations in platelet adhesiveness and aggregation to collagen most frequent, the severity of these defects was analyzed in relation to changes in IgM. Among the 30 patients in whom both platelet functions and immunoglobulins had been determined, platelet adhesiveness was subnormal in 19. In all but two of these, IgM was higher than 2 SD of its normal mean (Fig. 2). Likewise, as shown in Figure 3, there was only a single instance of poor platelet aggregation to collagen where IgM did not show an increase beyond 2 SD of its normal mean.

The results of aggregation of platelets to collagen following its incubation with leprosy and normal plasma are presented in

TABLE 3. *Immunoglobulin levels in patients of lepromatous and tuberculoid leprosy in mg% ± S.D.*

Immunoglobulins	Controls[a] (10)	Lepromatous leprosy[a] (23)	Tuberculoid leprosy[a] (7)
IgG	1486.0 ± 203.0	1272.0 ± 211.0	1311.0 ± 329.0
IgM	155.0 ± 20.0	253.0 ± 61.0	294.0 ± 22.0
IgA	288.0 ± 60.0	317.0 ± 25.9	316.0 ± 20.0

[a] Figures in parentheses represent number of patients in each group.

TABLE 4. *Percent aggregation to collagen of normal platelets after their incubation with normal plasma or plasma from patients having leprosy.*

Incubation with patient's plasma	Incubation with control plasma
14.3	43.0
14.2	43.0
23.0	43.0
60.7	35.7
10.7	35.7
10.0	50.0
20.0	50.0
17.5	50.0
60.0	50.0
47.5	50.0

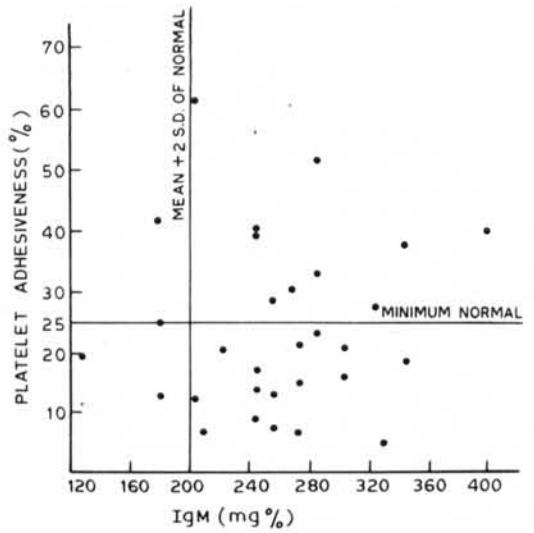

FIG. 2. Platelet adhesiveness in relation to IgM levels in the plasma.

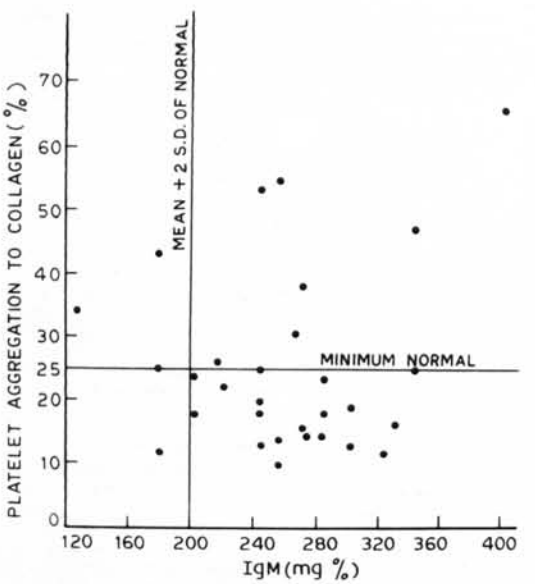

FIG. 3. Platelet aggregation to collagen in relation to IgM levels in the plasma.

Table 4. Whereas platelet aggregation was between 36% and 50% when collagen was incubated with normal plasma, it was 20% and below in as many as seven of ten instances where collagen had been incubated with patient's plasma.

DISCUSSION

This study reports hitherto unrecognized laboratory evidence of defective platelet function in leprosy patients. One or another platelet abnormality affected as many as 44 of the 50 patients. The most profound changes were observed in platelet adhesiveness and their aggregation to collagen.

Since alterations in immunoglobulins decidedly affect platelet function ([6, 15, 19]), the causative role of elevated IgM levels in causing platelet dysfunction in our patients appeared very probable. Indeed, in a large majority of the patients subnormal platelet adhesiveness and their decreased aggregation to collagen were associated with significantly raised IgM levels. Theoretically, two possibilities exist: impairment of platelet adhesiveness and aggregation may result from changes in platelet surface *per se*, or may be due to alterations in the aggregating surface such as collagen, or both. There is evidence to show that alterations in platelet function may result from coating of platelets by macroglobulins ([13]) and that a membrane abnormality in such platelets may be seen by the absence of dendritic formation electron microscopically ([5, 13]). This appears untenable in our patients. Had it been a change in the platelet surface it would have been reasonable to expect platelet aggregation to be impaired not only to collagen but to ADP as well. Furthermore, it would seem that the platelet coating would also interfere with the release of Pf-3. Diminished Pf-3 availability was, however, an infrequent feature in these patients. Alternatively, the increased IgM concentration in our patients could so alter the collagen that its clumping activity for platelets is diminished. This is borne out by the *in vitro* experiments in which incubation of collagen with plasma from leprosy patients diminished considerably its clumping ability for normal platelets. Vigliano and Horowitz ([19]) demonstrated diminished adhesiveness and aggregation of normal platelets to collagen when the latter had been incubated with the serum from a patient having IgA myeloma. These reasons led us to believe that in leprosy, impairment of platelet adhesion and aggregation to collagen results from coating of collagen by IgM.

In none of the patients did even multiple defects of platelet function result in clinical bleeding. It is possible that these defects were just severe enough to result in a hypocoagulable state of blood but not bleeding. If

so, the reported infrequent incidence of thromboembolic phenomenon in leprosy becomes explainable.

SUMMARY

In a group of 50 leprosy patients, platelet function tests were found to be abnormal in 44. More than half the patients showed significant impairment in platelet adhesiveness and aggregation to collagen which correlated best with increase in serum IgM levels. ADP-induced aggregation of platelets was not a major defect and Pf-3 availability was reduced only in a fourth of the patients. *In vitro* incubation of collagen with plasma from leprosy patients significantly reduced its ability to clump normal platelets. This appears to be the first report of defective platelet function in leprosy, and it is thought that such changes may in part be due to increased IgM globulins in the blood and/or to alterations in the collagen brought about thereby.

RESUMEN

Se encontró que las pruebas de función plaquetaria eran normales en 44 de un grupo de 50 pacientes con lepra. Más de la mitad de los pacientes mostraron alteraciones significativas de la adhesión y agregación al colágeno de las plaquetas, que se relacionaban mejor con un aumento de los niveles séricos de IgM. La agregación de plaquetas inducida por ADP no fué un defecto importante y la disponibilidad de Pf-3, se redujo sólo en una cuarta parte de los pacientes. La incubación *in vitro* de colágeno con plasma de pacientes con lepra, redujo significativamente su habilidad para aglutinar plaquetas normales. Este parece ser el primer informe sobre una función plaquetaria defectuosa en lepra, y se cree que estos cambios pueden deberse parcialmente a un aumento de las globulinas IgM en la sangre y/o a alteraciones del colágeno producidas por el mismo.

RÉSUMÉ

Parmi 50 malades de la lèpre, on a observé des épreuves anormales de fonctionnement des plaquettes chez 44 d'entre eux. Plus de la moitié des malades présentait une diminution significative de l'adhésivité des plaquettes et de leur aggrégation au collagène, qui présentait une corrélation avec une augmentation des taux sériques de l'IgM. L'aggrégation des plaquettes induites par l'ADP ne consitutait pas un défaut majeur. La libération de Pf-3 était réduite chez un quart des malades seulement. L'incubation *in vitro* de collagène avec du plasma recueilli chez des malades de la lèpre diminuait de façon significative la capacité du plasma de précipiter les plaquettes normales. Ceci semble être le premier travail relatant des troubles de la fonction des plaquettes dans la lèpre. On pense que ces modifications peuvent en partie être dûes à une augmentation du taux des globulines IgM dans le sang, ainsi qu'à des altérations du collagène qui peuvent en résulter.

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