Alternate Pathway Activation in Sickle Cell Disease and β-Thalassemia Major

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Total hemolytic complement activity (CH50), immuno-electrophoretic conversion of Factor B (C3PA), and of C3 were studied in 16 patients with sickle cell disease in a steady state, eight patients in crisis, and ten patients with β-thalassemia major anemia maintained on a constant transfusion regimen. Patients with sickle cell disease in a steady state have moderatley 56 (percent) depressed conversion of Factor B in addition to markedly decreased conversion of C3 in four of ten patients. One of the three sickle cell patients and two of the four thalassemia patients with low C3 conversion levels have died subsequent to the studies. The combination of chronically decreased Factor B conversion in the face of markedly decreased C3 conversion may make these patients occasionally vulnerable to overwhelming infection analagous to the situation seen in postsplenectomy cases.

Patients with sickle cell disease and β-thalassemia major anemia have long been recognized to have an increased propensity toward infections, especially those with encapsulated organisms such as the pneumococcus.[1-10] The reason for this increased incidence of severe infection is not clear although both groups of patients have several features in common. Both groups have reticuloendothelial blockade and eventual iron overload of the reticuloendothelial system.[11,13] Both groups of patients eventually undergo splenectomy, either autologous[12] or surgical.[14] Recent evidence in patients with sickle cell disease indicates that a defect in the alternate pathway of complement activation might be responsible for the tendency toward a higher incidence of infection.[15-17]

The "alternate pathway" is the term used to describe the activation of the third component of complement not utilizing specific preformed antibody and bypassing a requirement for fixation of the early complement components. The alternate pathway appears to be an important mechanism in natural immunity (ie, host defense in primary infection). Its function is not dependent on preformed antibody.[17,18] Animals and humans who are unable to activate the classic pathway, because of deficiency of early components, appear to be in good health implying complement activation by the alternate mechanism.[19-21] Decreased complement activation is felt to lead to decreased opsonization and the subsequent susceptibility to infection in these disease groups. In this study, patients with sickle cell disease in the steady state and in crisis and patients with β-thalassemia major were evaluated to determine if alternate pathway activation of complement was impaired, and, if so, whether this impairment could be correlated with the clinical state of the patient.

Patients

The patients with sickle cell disease fell into two groups. In the first group were patients with documented sickle cell disease who were clinically well, not infected, or in crisis. There were 16 patients in this group. Their characteristics are given in Tables 1, 2, and 3.

The second group contained eight patients, all of whom were in active crisis as defined previously.[22] All of the patients in this group were homozygous SS except patient 8 who had S β-thalassemia.

All patients with β-thalassemia were transfusion dependent and required transfusions every two to four weeks. They were all severely iron overloaded as documented by serum ferritin values (Table 4). Consent and approval were obtained for all studies.

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Table 1. Complement Levels and Conversion Data in Sickle Cell Patients in a Steady State

Patients	Age	Sex	CH_{50}	Conversion of Factor B*	Conversion of C_3**
A	24	M	109	51	100
B	21	M	99	100	100
C	28	M	115	33	90
D	30	M	115	66	100
E	32	M	122	38	72
F	20	M	72	50	100
G	10	M	91	38	100
H	24	M	124	50	100
I	12	M	82	12	87
J	45	F	81	6	55
K	28	F	103	66	100
L	26	M	31	88	90
M	24	M	131	66	84
N	29	M	115	51	50
O	27	M	106	100	90
P	29	M	121	80	90
Mean	26		101	56	88
SD	±8		±25	±30	±16

*Immunoelectrophoretic conversion of Factor B to Factor $\bar{B}$ used as a measure of the degree of activation of Factor B following incubation of serum with inulin (See Methods).
**Immunoelectrophoretic conversion of C_3 to C_{3i} used as a measure of activation of C_3 following incubation of serum with inulin.

Methods and Materials

Blood was drawn from each patient and allowed to clot. The sera were then separated from the clot and stored, within two hours, at –70 C until the time of testing. The sera were then thawed and placed immediately in an ice bath. Total complement levels (CH50) were determined by standard methods.[23,24] Assessment of alternate pathway integrity was determined by measurement of the immuno-electrophoretic conversion of C3 to Factor C3i and Factor B to Factor $\bar{B}$ as described by Gotze and Muller-Eberhard.[25]

Immunoelectrophoresis slides were read by one of the authors who did not know the clinical state of the patients. Results were given as a percent of normal control sera activation. Statistical comparison of the results was performed using Student's t-test or a one-tailed test between proportions.[26] Serum ferritin levels were determined by radioimmunoassay.[27]

Results

As documented in Tables 1-4, there were no significant differences between CH50 levels in the various groups studied or when compared to values for normal controls. Table 1 documents complement levels in sickle cell patients in the steady state. C3 conversion in this group was minimally depressed to 88 percent and conversion of Factor B was moderately depressed to 56 percent of that of normal controls. Depressed conversion of Factor B could not be correlated with the age of the patient or with other clinical sequelae of sickle cell disease.

Table 2 documents the complement levels in sickle cell patients in crisis. CH50 levels are slightly increased, reflecting the fact that CH50 activity is often an acute phase reactant. Conversion of Factor B and C3 were also depressed in this group as a whole to 46 percent and 58 percent, respectively. Of note is the significant depression of conversion of C3 in a subgroup of three patients. The decrease from 88 percent C3 conversion in patients not in crisis to 58 percent in patients in crisis is statistically significant at $p<0.02$ and is accounted for by the last three patients. Of concern is that of the last three patients, patient 7 died about one month following complement level determination. Death was attributed to sickle cell crisis and the patient did not appear to be infected at the time.

Table 3 gives the results on three patients who were studied while in crisis and in a steady state. There is no consistent trend in the conversion of Factor B or C3 between crisis and the steady state for any given patient or for the group as a whole. There is no difference between the mean conversion values in any instance ($p<0.05$).

Table 4 documents complement determinations in patients with β-thalassemia major. The mean age in this group of patients was somewhat younger than those with sickle cell disease owing to their decreased lifespan.[14] Six of the ten patients had undergone splenectomy at various time intervals preceding determination of complement conversion. That the patients were severely iron overloaded is documented by the mean serum ferritin level of 15,043 μg/100 ml for the group. CH50 levels were not decreased in this group of patients. Conversion of Factor B and conversion of C3, however, were both decreased to 29 percent and 59 percent of normal controls, respectively.

There is a subgroup of thalassemia patients with markedly decreased conversion of both Factor B and C3 (patients 4, 8, 9, and 10). They account for the total decrease in conversion of C3 with a mean level of 19 percent. The mean conversion of C3 for the remaining six patients is 87 percent. This subgroup also accounts for much of the decrease in conversion of Factor B, having a mean of 7 percent as opposed to 43 percent for the remaining six patients. The amount of conversion of Factor B and C3 could not be correlated with the age of the patient, the amount of iron overload as determined by serum ferritin, or the clinical status of the patient. Of note, however, is that two of the four patients with the lowest conversion values (patient 4 and patient 10) have died within two years of the determination of these values. Both patients died suddenly before they could reach the hospital. Patient 4 had a high fever prior to her death. The cause of death in patient 10 could not be determined. Although the four patients with the lowest conversion values for Factor B and C3 all were status post-splenectomy, patients 1 and 3 were also postsplenectomy but did not have mark-

Table 2. Complement Levels and Conversion Data in Sickle Cell Patients in Crisis

Patient	Age	Sex	CH_{50}	Conversion of Factor B*	Conversion of C**
1	28	M	119	66	100
2	17	F	99	100	100
3	17	M	49	35	83
4	24	M	128	25	50
5	29	M	110	31	83
6	19	F	157	29	15
7	28	F	69	29	15
8	21	F	170	50	15
Mean	23		113	46	58
SD	5		41	26	39

*Immunoelectrophoretic conversion of Factor B to Factor $\bar{B}$ used as a measure of the degree of activation of Factor B following incubation of serum with inulin (See Methods).
**Immunoelectrophoretic conversion of C_3 to C_{3i} used as a measure of activation of C_3 following incubation of serum with inulin.

Table 3. Conversion Data on Sickle Cell Patients in Crisis and in a Steady State

Patient	Age	Conversion of Factor B*		Conversion of C_3**	
		Crisis	Steady State	Crisis	Steady State
A	28	66	33	100	90
B	24	25	66	50	84
C	29	31	51	83	50
Mean	27	41	50	78	75
SD	3	22	17	25	22

*Immunoelectrophoretic conversion of Factor B to Factor $\bar{B}$ used as a measure of the degree of activation of Factor B following incubation of serum with inulin (See Methods).
**Immunoelectrophoretic conversion of C_3 to C_{3i} used as a measure of activation of C_3 following incubation of serum with inulin.

edly depressed C3 or Factor B conversion.

Discussion

The alternate pathway of complement activation involves activation by a mechanism other than classic activation via antigen antibody complexes. Activation of C3 by either the classic or alternate pathway produces its derivative C3b which can trigger a feedback amplification system in which more C3 convertase is produced by the interaction of C3b. C3b deficiency, through whatever mechanism, would lead to a defect in opsonization.[15-17]

The present study confirms a moderate decrease in conversion of Factor B in patients with sickle cell disease and in patients with β-thalassemia major. Whether this was the primary defect which predisposes these patients to overwhelming sepsis could not be determined.

Of note is a subgroup of patients with sickle cell disease in crisis and with β-thalassemia major anemia who had marked impairment in conversion of C3 in addition to depressed conversion of Factor B. Of further note in this subgroup of patients is the fact that of seven patients, three have died in the two years following determination of complement activation. There were no deaths among the 24 patients who did not show decreased conversion of C3. This makes the probability of three deaths in the subgroup of seven highly unlikely ($p<0.001$). It would appear, then, that decreased conversion of Factor B provides a background against which an acute depression of conversion of Factor C3 makes these patients highly vulnerable and conveys a poor prognosis.

The role of various factors which might predispose to this situation could not be determined from the present study. All the patients with sickle cell disease were of an age where functional asplenia would be expected.[12,13] All the patients with β-thalassemia major were severely iron overloaded and would be expected to have reticuloendothelial blockade, as would the patients with sickle cell disease.[12,13]

The data are consistent with recent studies on patients undergoing abdominal surgery and splenectomy. Patients undergoing splenectomy showed a total decrease in C3 conversion which was significantly lower than postoperative controls. Patients undergoing splenectomy were able to activate C3 more rapidly five months postsplenectomy despite the fact that levels in most cases remained depressed when compared to those in the normal controls. The time course indicated that the maximum vulnerability to infection postsplenectomy was within five months of the operation although individual patients showed depressed activation of C3 for periods of longer than five months.[28]

There was also a discrepancy between activation of Factor B and activation of C3 in splenectomized patients. The ability to activate Factor B in postoperative patients and postsplenectomy patients was diminished to about the same extent. On the other hand, activation of C3 was diminished to a much greater extent postsplenectomy than in the postoperative control group. This indicates that postsplenectomy vulnerability to infection is accompanied by a moderate decrease in Factor B but markedly decreased conversion of C3. The discrepancy between C3 activation and Factor B activation indicates that C3 conversion may be dependent on other means of activation than Factor B. This is consistent with recent data that point to several means by which C3 conversion may be achieved.[28]

The series of events which leads to a depression in conversion of C3 in the presence of an already compromised conversion of Factor B requires further

Table 4. Complement Determination and Conversion Data in Patients with β-Thalassemia Major

Patient	Sex	Age	Age Splenectomy	Serum Ferritin (μgm/100 ml)	CH_{50}	Conversion of Factor B*	Conversion of C_3**
1	F	20	12	13,560	NA	44	100
2	F	9	—	16,230	94	38	90
3	M	14	5	22,242	102	55	54
4	F	13	8	11,040	47	5	29
5	M	13	—	14,730	110	40	92
6	M	13	—	16,747	126	60	100
7	M	10	—	NA	75	20	83
8	F	18	3	12,145	88	9	10
9	M	23	5	13,650	121	9	30
10	F	19	9	NA	148	5	5
Mean		15		15,043	101	29	59
SD		±5		±3,481	±30	±21	±38

NA — Not available
*Immunoelectrophoretic conversion of Factor B to Factor $\bar{B}$ used as a measure of the degree of activation of Factor B following incubation of serum with inulin (See Methods).
**Immunoelectrophorectic conversion of C_3 to C_{3i} used as a measure of activation of C_3 following incubation of serum with inulin.

study. Nevertheless, patients with decreased splenic function, whether through surgical removal or autosplenectomy, appeared to be more vulnerable to this series of events. Whether penicillin prophylaxis is warranted in the subgroup of patients with depressed conversion of C3 and Factor B remains to be determined.

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