

CHAPTER III

RESULTS



Antipenicillin antibody detection

Results of the antipenicillin antibody detection in the sera of patients' group as well as the group of healthy individuals donating blood are shown in Table 3. Circulating antipenicillin antibody were found in 96 of 2500 unselected patients (3.8%) and 3 of 70 patients (4.3%) with cardiovascular disease who had recently receiving penicillin. None of the 230 healthy individuals donating blood had antipenicillin in their sera.

The serologic studies of circulating antipenicillin antibody with penicillin-treated red cells, at room temperature, 37°C and antiglobulin test are shown in Table 4. Of the total 99 sera positive for antipenicillin antibody detections, 3 (8.1%) were detectable at room temperature and after incubation at 37°C for 30 minutes, 66 (66.6%) were detectable at all three phase tests. Twenty-five of the 99 sera (25.3%) had antipenicillin antibody detectable only by the antiglobulin technique.

Antimethyldopa antibody detection

Thirty-two hypertensive patients on treatment with methyldopa, 11 patients were male and 21 were female. The patients were divided

Table 3 Results of detectable antipenicillin antibody in the sera of patients and healthy donors.

Group	No. sera tested	No. positive sera	Positive sera %
Unselected patients	2500	96	3.8
Patients with cardiovascular disease	70	3	4.3
Healthy donors	230	0	0

Table 4 Serologic studies of antipenicillin antibody with penicillin-treated red cells.

Serologic studies of antibodies			No. sera tested	No. positive sera	positive sera %
room temp.	37°C	AGT*			
+	+	-	99	3	3.1
+	+	+	99	66	66.6
-	-	+	99	25	25.3

* Antiglobulin test.

into three groups according to dosage of methyldopa : -11 patients taking 0.50 g or less per day; 13 patients taking 0.75 g per day; and 8 patients taking 1 g or more per day.

The sera of all 32 patients when tested with normal red cells and methyldopa solution failed to show hemolysis or agglutination. The sera of 32 patients did not contain agglutinating antibody at any concentration of methyldopa or serum dilution up to 1:128.

Immunoglobulin classes of circulating antipenicillin antibody

In order to determine the immunoglobulin classes of circulating antipenicillin antibody, sera were inhibited with 2-Mercaptoethanol. Of 99 patients' sera, 31 (31.3%) were completely inhibited with 2 ME, i.e., they contained only IgM antibodies. Fifty-three (53.5%) contained mainly IgM plus IgG antibodies, i.e., they had comparatively lower titers of IgG antibodies (1:2 to 1:128) as well as higher titers of IgM antibodies (1:8 to 1:1024). They had IgM in addition to IgG antibodies. Sixteen (16.2%) were completely or mainly 2 ME resistant, they contained mainly IgG antibodies (Table 5).

The distributions of antipenicillin antibody titers of these patients are shown in Figure 4. The mean titers were 1:8 for IgM, 1:64 for IgM in addition to IgG and 1:128 for IgG antibodies.

Table 5 Immunoglobulin classes of circulating antipenicillin antibody

Immunoglobulin classes	Antipenicillin antibody titers	No. sera tested	No. positive sera	positive sera %
IgM	1:2 to 1:32	99	31	31.3
IgM plus IgG	1:8 to 1:1024	99	52	52.5
IgG	1:32 to 1:512	99	16	16.2

No. of Patients.

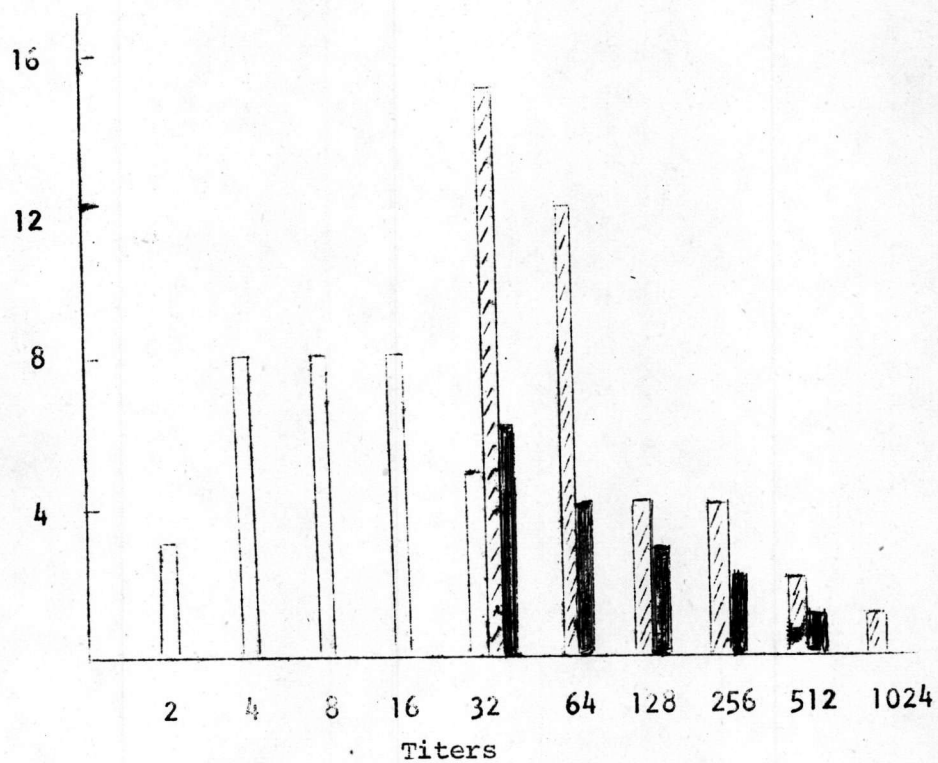


Figure 4 Antipenicillin antibody titers of 99 patients.

Open bar = titers of IgM antibodies, diagonal

/ bar = titers IgM in addition to IgG antibodies,

and solid bar = titers of IgG antibodies.

Incidence of a positive direct antiglobulin

Results of positive direct antiglobulin test of 99 patients having antipenicillin antibody in their sera are shown in Table 6. Using a broad spectrum antiglobulin sera, a positive direct antiglobulin test was found in 4 of 96 unselected patients (4.2%) and one in 3 patients (33.3%) with cardiovascular disease. One patient with cardiovascular disease, was only one of 45 patients (2.2%) who had recently received complete course of penicillin therapy after cardiac surgery.

Using monospecific antisera, the red blood cells of 5 patients gave a positive reaction with anti-IgG serum. When tested with anti-complement serum the direct antiglobulin test was negative in all of them (Table 7).

Eluates were prepared from the red blood cells of 5 patients having positive direct antiglobulin test, reacted with penicillin-treated red cells only by indirect antiglobulin technique. None of these eluates reacted with non-treated red cells, and they also gave negative reaction when tested against pooled panel red cells. These findings indicated that the antibodies that coated or adsorbed on patient's red blood cells were antipenicillin antibody.

Results of the characteristics and titers of sera and eluate antibodies of 5 patients are shown in Table 8. The antipenicillin antibody titers before and after treatment with 2 ME of 4 patients, were 256/256, 1024/512, 128/128 and 128/64, suggesting the presence of mainly IgG antibodies. Only one patient containing IgM in addition to IgG antibodies. In all 5 eluates indicating the presence

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Table 6 Incidence of a positive direct antiglobulin test in patients having antipenicillin antibody.

Patient's group	No. of patients	No. positive direct AGT
unselect patients	96	4 (4.2%)
patient with cardiovascular disease	3	1 (33.3%)
Total	99	5 (5.1%)

Table 7 Results of a direct antiglobulin test with specific antisera in 5 patients

Patient No.	Antiglobulin reaction using		
	B.S. *	Anti-IgG	Anti-complement
1 st	2+	2+	-
2 nd	2+	1+	-
3 rd	1+	1+	-
4 th	1+	1+	-
5 th	1+	1+	-

* Broad spectrum antiglobulin serum

of IgG antipenicillin antibodies as shown by resistant to 2 ME .
inhibitions.

Hematologic, clinical and other laboratory data of the patients who had antipenicillin antibodies in their sera and developed a positive direct antiglobulin test are shown in Table 9. In all 5 patients had recently receiving penicillin parenterally for 3 to 7 days in doses ranging from 1.2 million units to 12 million units per day. Antipenicillin antibody detection and direct antiglobulin test were done between 3 to 24 days after penicillin therapy had been discontinued.

One patient was anemic, having a hemoglobin of 4.7 g%, in this case the course of anemia was apparently due to the underlying disease Thalassemia HbE. Three patients were mildly anemic had a hemoglobin of 10.0, 10.5 and 11.4 g% respectively. Only one patient , had hemoglobin in a normal range 13.2 g% .

Results of the incidence of a positive direct antiglobulin test in patients receiving methyldopa are shown in Table 10. Three of 32 patients (9.4%) who were taking methyldopa had a positive direct antiglobulin test. Of the 24 patients with hypertension who had receiving other drug (i.e., reserpine), none of this control group had a positive direct antiglobulin test.

Table 8 Characteristics and titers of antibodies from sera and eluate of five patients having a positive direct antiglobulin test.

Patient No.	Source of antibodies	Antipenicillin antibody titers*		
		R.T.**	37°C	AGT
1 st	serum	4/0	4/0	256/256
	eluate	0/0	0/0	8/4
2 nd	serum	512/128	512/128	1024/512
	eluate	0/0	0/0	2/2
3 rd	serum	0/0	0/0	128/128
	eluate	0/0	0/0	4/4
4 th	serum	2/0	2/0	128/64
	eluate	0/0	0/0	2/2
5 th	serum	16/0	16/0	64/4
	eluate	0/0	0/0	4/2

* Before/after 2 ME inhibition.

** Room temperature

Table 9 Hematologic, clinical and other laboratory data of five patients who had circulating antipenicillin antibody and developing a positive direct antiglobulin test.

Patient No.	Penicillin dose/day (million units)	Duration of therapy (days)	CAPA titers	Direct AGT	Hb g%	Clinical conditions
1 st	12	6	1:256	2+	4.7	Thalassemia with fever
2 nd	1.2	5	1:1024	1+	11.4	Multiple injury with bleeding
3 rd	12	2				
	12	7	1:128	1+	10.5	Septicemia
4 th	12	3	1:128	1+	10.4	Cirrhosis of liver
5 th	12	4	1:64	1+	13.2	Constrictor pericarditis

Table 10 Incidence of a positive direct antiglobulin test in hypertensive patients

Patients	No. tested	No. with positive direct AGT
receiving methyldopa	32	3 (9.4%)
not receiving methyldopa	24	0

Results of direct antiglobulin test with anti-IgG and anti-complement are shown in Table 11. There is no evidence of any complement on the patient's red cells, the reaction is of the anti-IgG antibodies.

The eluates were prepared from red blood cells of 3 patients who developing positive direct antiglobulin test gave negative reaction when tested against pooled panel red cells for irregular blood group antibodies detection.

Attempts were made to show the anti-methyldopa antibody in patient's sera and eluates by incubating with normal red cells and methyldopa solution room temperature, and 37°C, followed by antiglobulin technique. Sera and eluates failed to show agglutination or hemolysis. These findings indicated that both sera and eluates from patient's red cells did not contain any irregular blood group

antibodies and no evidence of antibody to methyldopa.

Table 12 shows the hematologic and other laboratory data of three patients who had a positive direct antiglobulin test. Two patients had been taking methyldopa 0.75 to 1 g per day, and one patient 1.5 g per day. Two patients were on methyldopa before study less than 6 months, one patient was more than 6 months (3 years).

All of the 3 patients were mildly anemic, the hemoglobin were 10.3 g%, 9.7 g% and 10.0 g% respectively. Two patient had reticulocyte count within the normal range (0.1-1.5%). Blood urea nitrogen (BUN) was elevated to 61 mg%, 40 mg% and 54 mg%, respectively.

Summary of the incidence of a positive direct antiglobulin test in hypertensive patients in comparison with results reported previously by various authors are shown in Table 13.

Table 12 Hematologic, chemical and immunohematologic studies of three patients taking methyldopa who had a positive direct antiglobulin test.

Patient No.	Strength of direct AGT	Hemoglobin (g%)	Reticu- locyte count (%)	Bili rubin mg%	BUN mg%
1 st	2+	10.3	ND	ND	61
2 nd	2+	9.7	1.2	0.9	40
3 rd	2+	10.0	1.2	0.6	54

Table 13 Incidence of a positive direct antiglobulin test in hypertensive patients
(summary of reported cases).

Reference	No. tested	Methyl dopa & other drugs		Other drugs	
		No. with positive direct AGT	No. with overt hemolysis	No. tested	No. with positive direct AGT
<u>Contor et al (67)</u>	55	8 (14.5%)	0	25	0
<u>Cotton et al (55)</u>	45	4 (9%)	0	-	-
<u>Feiji et al (75)</u>	50	2 (4%)	0	50	0
<u>Logue et al (66)</u>	47	6 (10.6%)	1	-	-
<u>Seedat et al (76)</u>	75	0	0	-	-
<u>Weiner et al (77)</u>	26	4 (15%)	0	41	0
<u>Worlledge et al (62)</u>	572	115 (20%)	2	100	0
<u>Wurzel et al (64)</u>	31	5 (16%)	0	12	0
Present report	32	3 (9.4%)	0	24	0