NON-SPECIFIC FIXATION OF COMPLEMENT: THE ANTICOMPLEMENTARY ACTIVITY

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Abstract

A cause and effect relationship of hepatitis-B antigen-antibody complexes, temperature and the anticomplementarity of the stored sera was studied in 14 antigen-positive and 373 antigen-negative sera. Our findings on anticomplementary activity of the stored sera suggest that the anti-complementary activity was not due to HB-antigen-antibody complexes or at least not due to complexes which were dissociable by an excess of hepatitis-B antigen. On the other hand the temporal relation between anticomplementary activity and storage of sera for variable length of time suggests that the anticomplementary activity was due to repeated cycles of thawing and freezing.

Introduction

Complement fixation test is among the most frequently used serological methods; it is simple and highly satisfactory and can be employed with either soluble or particulate antigen for detection either of antigen or antibody.

One of the important problems associated with complement fixation tests is that of anti-complementary (AC) activity of the serum. It is well recognised that low dilutions of serum tend to fix complement non-specifically and many sera show anticomplementary activity at a dilution of 1:2; for this reason complement fixation tests are generally performed on sera diluted at various dilutions (Schmidt and Lennette, 1970). The anticomplementary activity that may develop in normal serum kept at room temperature for a few days present no problem as it can be easily eliminated by heating at 56°C for 30 minutes (Cruickshank 1965).

There is a general agreement that anticomplementary activity is probably caused by immune complexes in the circulation. However, there is disagreement as to the nature of these complexes. Thus Shulman and Barker (1969) demonstrated that 95 percent of patients having hepatitis associated with blood products developed anti-complementary activity in their sera at some time during the course of hepatitis, with anti-complementary titers from 1:4 to 1:512. They found that the activity could be reversed by preliminary incubation with excess hepatitis-B antigen (HBAg) or antibody and suggested that the activity might be due to complexes of hepatitis-B antigen and antibody in the sera. Purcell et al (1970) also observed anticomplementary activity in the sera of hepatitis patients, but in low proportion of patients (5 out of 32) and in low titers (1:2 to 1:16). On the other hand Schmidt and Lennette (1970) and Zuckerman (1969) have demonstrated that anticomplementary (AC) activity may be due to factors other than hepatitis-B antigen antibody complexes. Purcell et al (1971) have also reported that anticomplementary activity may be caused by any antigen antibody reaction and the individual with sera showing anticomplementary activity should not be considered as having hepatitis B unless hepatitis-B antigen can be specifically demonstrated in the complexes.

Storing the sera at a temperature more than-20°C also results in anti-complementary activity (Bradstreet and Taylor, 1962) and recently Lander et al (1972) have reported the anticomplementary activity of most of the sera as a result of numerous cycles of thawing and freezing. Since most of the stored sera in our study on the "Prevalence of Antibodies against Australia antigen in General Population" showed anticomplementary activity (Khadim 1977), it was considered worthwhile to study the relationship of the anticomplementary activity with antigen-antibody complexes and temperature and to see whether one or both were the responsible factors.

Material and Methods

Stored Sera

Stored sera from 250 apparently healthy Naval recruits and 250 house hold contacts of hepatitis patients were obtained from the Research Centrel, Pakistan Medical Research Council, Jinnah Postgraduate Medical Centre, Karachi. None of these individuals had evidence of liver disease, however, 13 of the contacts and 7 of the recruits were positive for hepatitis-B antigen. The serum samples from the recruits and the contacts were kept frozen at-20°C for 2 years and I year respectively and had undergone thawing at different intervals before the commencement of the present study.

Fresh Sera

In order to compare the anti-complementary activity, fresh sera were obtained from the clinical laboratories and the P.M.R.C. Research Centre, Jinnah Postgraduate Medical Centre, Karachi, between 7-2-1977 and 20-2-1977 daily or at one or two days interval from 125 patients (other

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than hepatitis) and stored (for about a month) at-20°C till analysed.

The complement fixation test was performed by the microtiter methods as described by Bradstreet and Taylor (1962). All the sera were heated undiluted at 56°C for 30 minutes to destroy the endogenous complement. Sera were tested at dilutions of 1:2-1:256. Three units of complement were used. The serum, antigen/diluent and complement were mixed and incubated overnight at 4°C; the sensitized sheep cells were then added. The test was performed with acrylic microtiter plates and 0.025 ml droppers.

Results

Three hundred and seventy three of 480 individuals without hepatitis-B antigen and 14 of 20 individuals with hepatitis-B antigen had anticomplementary (AC) serum specimens. As shown in table I the AC titers of the sera were generally between 1:2 through 1:128 and the highest dilution at which most of the sera (22.5%) showed AC activity was 1:16.

same extent. Treatment was conducted without incubation prior to the addition of complement and with a preliminary incubation period of 2 hours at 37°C. As indicated in table II, the 1:16 Ac titers of all the sera remained unchanged by treatment with excess antigen.

Table III: Temporal Relation of AC Activity and Duration of the Stored Sera

Duration of stored sera	*No. of plsera tested*	*Anticomplementary activity*	*Percentage*
2 Years	250	228	91.2%
1 Year	250	159	63.6%
1 month	125	23	18.4%

To study the effect of thawing and freezing the AC activity in sera collected two years ago one year before and one month before the commencement of the present study, was compared and a temporal relation was observed. As given in table III, the sera which were collected earlier, stored for a longer period and had undergone thawing and freezing at different intervals,

Table I: Distribution of Anticomplementary (AC) Activity in 500 Sera According to AC Titer

Groups	*No. Serum samples tested*	*Sera with AC Activity*	*NO. OF SERA WITH AC TITERS*						
			1:2	1:4	1:8	1:16	1:32	1:64	1:128
HB Ag+ve	20	14	6	3	1	3	0	1	0
HB Ag—ve	480	373	50	93	147	55	21	5	2
Total:	500	387	56	96	148	58	21	6	2
						22.5%			

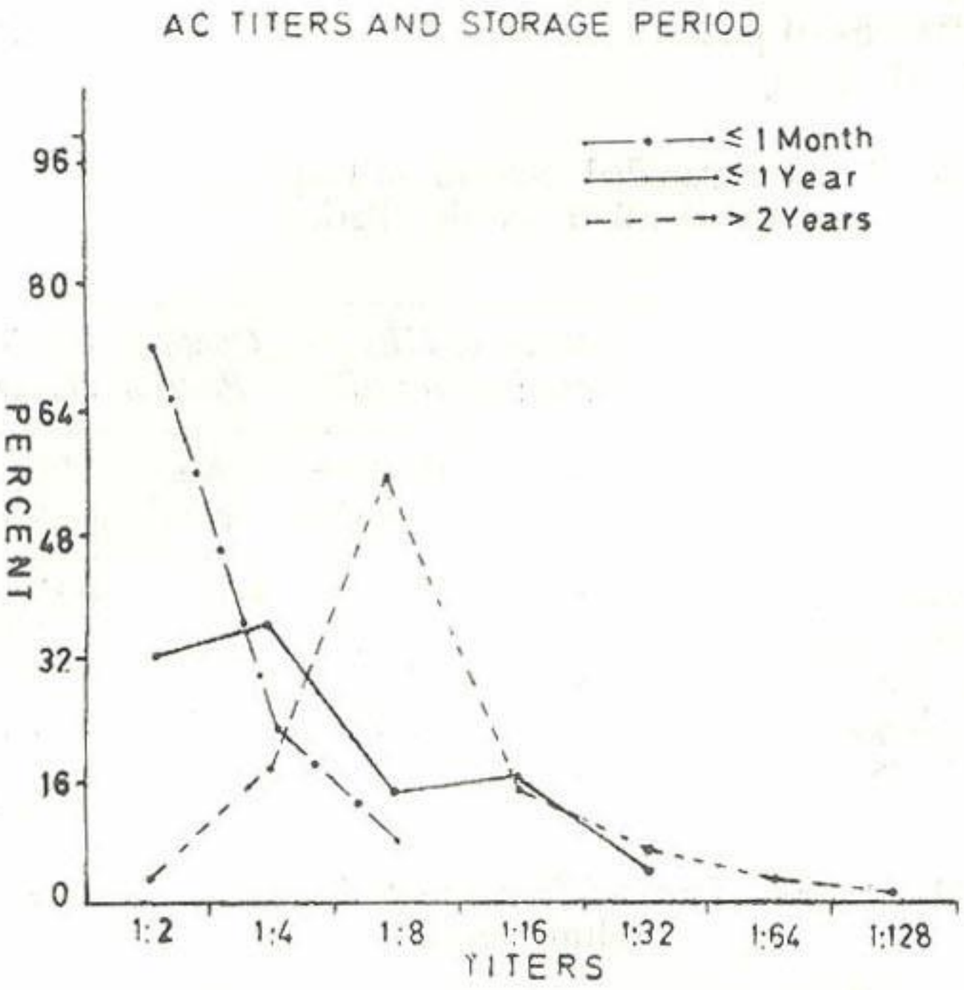
Table II: Effect of an Excess of HBAntigen and of Negative Serum on AC Activity

Serum Examined	*HB Antigen*	*AC Titer of Serum*	*AC TITER OF SERUM*					
			No incubation			*Incubated at 37°C 2 hours before complement is added.*		
			Antigen[a] excess	*Negativeb serum* 1	*Negative[2] serum*	*Antigen excess*	*Negative serum*	*Negative serum*
3	+	16	16	16	16	16	16	16
55	—	16	16	16	16	16	16	16

(a) Serum with concentration 1:1 to contain 16 units of HB antigen.
(b) Negative serum at concentration 1:1.
AC Titer of serum has been expressed as reciprocal of titer.

Attempts were made to study the reversibility of anticomplementary activity of the sera by treatment with an excess of antigen, and to see the effect of thawing and freezing on AC activity by matching the activity in old and fresh sera. To see the effect of excess antigen, three HB Ag-positive and fifty five HB Ag negative sera were treated in parallel with a dilution to the

showed more anticomplementary activity. The titer also increased with the passage of time reaching its peak level in sera (Figure). The highest titer at which most of the sera showed anticomplementary activity in 2 years, one year and one month stored sera were 8, 4 and 2 respectively, and exhibited statistically significant differences ($P < .01$ and $P < .001$).



Discussion

Our findings on anticomplementary activity of the stored sera (77.4%) are nearly similar to those reported by Shulman and Barker (1969). However, the anticomplementary activity in our study could not be reduced by treatment with an excess of hepatitis-B-antigen. These results suggest that the anticomplementary activity was not due to hepatitis-B antigen antibody complexes or at least not due to complexes which were dissociable by an excess of hepatitis-B antigen. On the other hand the temporal relation between anticomplementary activity and storage of sera for variable lengths of time suggests that the longer the sera are stored, the greater is the anticomplementary activity. One possible explanation for this in this study could be the repeated thawing and freezing of the stored sera which is suspected to have occurred because of some technical problems. The activity occurring characteristically during the year of storage, could be due to frequent use of sera for different tests at various intervals or frequent defrosting of the freezer causing increase ($\succ 20°C$) and decrease in temperature resulting in repeated thawing and freezing. The increase in titer with the time of storage indicates that anticomplementary activity is induced more with the passage of time. Anticomplementary activity in 12 percent of a set of 240 sera has also been reported by Schmidt and Lennette (1970) although no mention was made of the duration of storage.

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