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Relationships between the haemolytic activities of the human complement system and complement components

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SUMMARY

The relationships between the haemolytic activities of complement and its components were studied. The activities studied included CH50 (classical pathway), AP50 (alternative pathway), CV50 (early part of alternative pathway) and C(3–9)H50 (the late part of both pathways). The components included C3, C4, C5, C9, $\bar{B}$ and $\bar{D}$.

There was a good correlation between CH50 and AP50. AP50 had a good correlation with $\bar{B}$ and CV50. There was no correlation between AP50 and C(3–9)H50, and none between C(3–9)H50 and C5 or C9.

AP50 may primarily represent changes in the early part of the alternative pathway. C(3–9)H50 is not influenced by respective changes in the amounts of C5 or C9. Since cell lesion is now considered to be caused by a unit of C5b to C9, a change in each component of C5 to C9 may not influence haemolytic activity.

INTRODUCTION

The complement system is classified into two pathways, the classical and the alternative. Since Pillemer *et al.* (1954) introduced the properdin system as a humoral mechanism of resistance to infections, much has been written about the alternative pathway (Prahl, 1976). CH50 has been used as an indicator of the classical pathway and amounts of complement components, such as C3, C4, C5 and C9, have been measured by using a radial immunodiffusion technique. As an indicator of the activity of the alternative pathway, CVFHA50 was introduced by Brai & Osler (1972) by using cobra venom factor. $\bar{B}$ has been measured by a radial immunodiffusion technique and $\bar{D}$ haemolytic activity estimated by a haemolytic diffusion plate assay (Martin *et al.*, 1976). In 1974, Platts-Mills & Ishizaka reported that rabbit red blood cells (RRBC) directly activated the alternative pathway of human complement system. The present authors have introduced AP50 as an indicator of the activity of the alternative pathway by using RRBC (Takada *et al.*, 1978). AP50 was defined as the dilution of human serum to give 50% lysis of RRBC. Since the classical and alternative pathways have later components in common, AP50 may represent not only the early components of the alternative pathway (IF, properdin, B and D) but also its later components (C3–C9).

In the present research, we compared CH50, AP50 with other activities, such as CVFHA50 (abbreviated to CV50) and C(3–9)H50, in addition to amounts of various complement components.

MATERIALS AND METHODS

Human serum. Blood was taken from healthy adult male volunteers, and sera were kept at −70°C.

CH50. CH50 was measured according to Mayer (1961).

AP50. Haemolysis of RRBC by human serum was used. Serum (0·4 ml) serially diluted with EGTA-VB (veronal buffered saline, pH 7·5, containing 0·02 M Mg^{++} and 0·008 M ethylene glycol bis-amino tetraacetic acid) was mixed with 0·2 ml of

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RRBC (3×10^7) and incubated for 30 min at 37°C, and 4·4 ml of EDTA-GVB (veronal buffered saline containing 0·01 M ethylene diamine tetraacetate and 0·1% gelatin) was added. After centrifugation at 450 *g*, OD_{412} of the supernatant was measured. Dilution of serum to give 50% lysis of RRBC was tentatively called AP50 (Takada *et al.*, 1978; Tåkada & Takada, 1978).

Preparation of complement components and EAC142. The first component of guinea-pig complement (C1gp) was prepared according to the method of Nelson *et al.* (1966). The second (C2hu) and fourth (C4hu) components of human complements were prepared according to the method of Vroon, Schultz & Zraco (1970). Oxidation of C2hu was performed following the method of Polley & Müller-Eberhard (1967) and was referred to as oxyC2hu. EAC1 were prepared by reacting EA with C1gp (100 SFU/EA), and EAC14 were prepared by reacting EAC1 with C4hu (200 SFU/EAC1). Finally, EAC142 were prepared by incubating EAC14 with oxyC2hu (100 SFU/EAC14).

C(3–9)H50. C(3–9)H50 was measured by using EAC142. 0·2 ml sera serially diluted with EDTA-GVB and 0·1 lm of EAC142 (2×10^7) was mixed and incubated for 60 min at 37°C, then 4·0 ml of EDTA-GVB was added. After centrifugation at 450 *g*, OD_{414} of the supernatant was measured.

CV50. CV50 was determined by using a modified method of Brai & Osler (1972). 0·2 ml of cobra venom factor (CVF) from Naja naja (20 u) and 0·2 ml of serum diluted serially with EGTA-VB were mixed and incubated for 30 min at 37°C, then 0·2 ml of out-dated human plasma diluted 5 times with 0·1 M EDTA-VB (veronal buffered saline containing 0·1 M EDTA) and 0·4 ml of guinea-pig erythrocytes ($1·5\times10^7$, washed three times with 0·02 M EDTA-VB) were added. After incubation at 37°C for 60 min, 2·0 ml of saline was added. OD_{414} of the supernatant was measured after centrifugation at 450 *g*.

$\bar{D}$. This was determined according to the method of Martin *et al.* (1976). 1% agarose solution in EGTA-VB was mixed with 0·5 ml of guinea-pig erythrocytes ($2·5\times10^8$) and 0·25 ml of RD (serum without D), and incubated for 10 min at 43°C. An agar plate was made and a small hole punched. 5·0 μl of serum was put into the hole and incubated for 4 hr at 37°C, then the lysis area of the erythrocytes was measured.

$\bar{B}$, *C3*, *C4*, *C5 and C9.* Measurement of these components was done by using radial immunodiffusion plates (Hoechst).

RESULTS

Relationships between AP50 and $\bar{B}$, $\bar{D}$ or C(3–9)H50

Fig. 1 shows relationships between AP50 and $\bar{B}$ or $\bar{D}$. $\bar{B}$ had a better correlation with AP50 ($r = 0·73$) than $\bar{D}$ ($r = 0·39$). The regression curve between AP50 and $\bar{B}$ is expressed as $y = 0·47\chi+5·21$, and that between AP50 and $\bar{D}$ as $y = 0·05\chi+8·90$. This indicates that a unit increase in $\bar{B}$ results in a larger increase in AP50 than does a unit increase in $\bar{D}$. The amount of $\bar{B}$ in serum may influence the haemolytic activity of the alternative pathway more than the amount of $\bar{D}$.

Fig. 2a shows that there was a good correlation between CH50 and C(3–9)H50. Fig. 2b shows that there was no correlation between AP50 and C(3–9)H50. This result indicates that AP50 may represent early parts of the alternative pathway.

Relationships between CV50 and $\bar{B}$ or $\bar{D}$

Cobra venom factor (CVF) is considered to form a complex with B, and $\bar{D}$, which is a protease in the

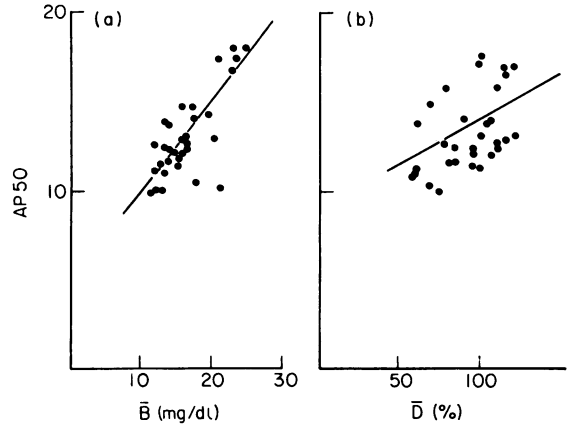

FIG. 1. (a) Relationship between AP50 and $\bar{B}$. (b) Relationship between AP50 and $\bar{D}$. Ordinate indicates unit of AP50 in both figures. Abscissa indicates the amount of $\bar{B}$ (mg/dl) in (a) and that of $\bar{D}$ in (b). The amount of $\bar{D}$ is expressed as a percentage of $\bar{D}$ in normal mixed serum.

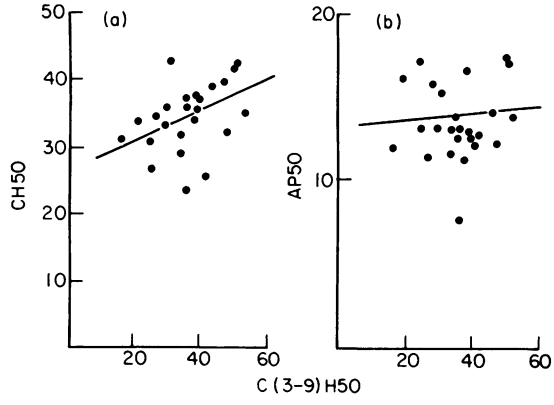

FIG. 2. (a) Relationship between CH50 and C(3–9)H50. (b) Relationship between AP50 and C(3–9)H50. Ordinate indicates unit of CH50 in (a) and unit of AP50 in (b). Abscissa indicates unit of C(3–9)H50.

serum, converts B to $\bar{B}$ in the complex. CVF-$\bar{B}$ complex functions as C3 convertase (Müller-Eberhard & Fjellstrom, 1971; Hunsicker, Ruddy & Austen, 1973). CV50 is measured by adding CVF to test serum, and then EDTA-treated plasma is added as a source of C3–C9. The amounts of C3 convertase generated in the first mixture of CVF and serum depend upon the amounts of B and D in the serum.

Fig. 3 shows the results of relationships between CV50 and $\bar{B}$ or $\bar{D}$. CV50 has a good correlation with $\bar{B}$ and $\bar{D}$. An increase in the amounts of $\bar{D}$ in the serum results in large increase in CV50. This indicates that $\bar{D}$ influences the haemolytic activity caused by CVF more than $\bar{B}$ does.

Correlation among haemolytic activities and complement components

Table 1 shows the correlation between CH50, AP50, CV50, C(3–9)H50 and complement components or other activities. Relationships with a correlation coefficient (r) larger than 0·5 were CH50/AP50, CH50/C3, CH50/C5, AP50/$\bar{B}$, and CV50/$\bar{D}$. There was no correlation between AP50 and C9, AP50 and C(3–9)H50, C(3–9)H50 and C5 or C9. With regard to the relationship between the classical and alternative pathways, CH50 had some correlation with $\bar{B}$.

DISCUSSION

Haemolysis of sensitized erythrocytes (EA) by components of the classical pathway of the human complement system has been used as an indicator of the activity of the classical pathway which was

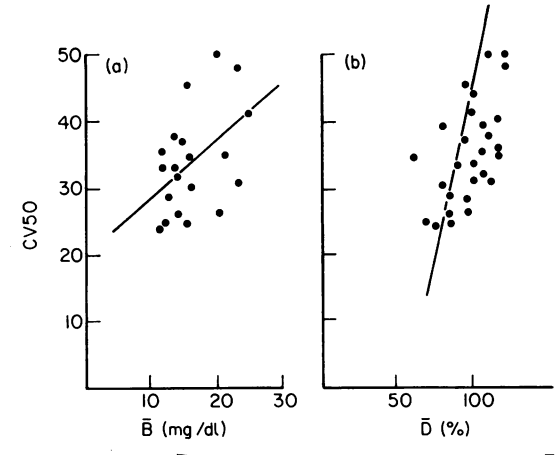

FIG. 3. (a) Relationship between CV50 and $\bar{B}$, and (b) relationship between CV50 and $\bar{D}$. Ordinate indicates unit of CV50. Abscissa indicates the amount of $\bar{B}$ (mg/dl) in (a) and that of $\bar{D}$ (%) in (b).

TABLE 1. Relationships between various parameters of the classical and alternative pathways

		r	P	n
CH50	AP50	0·68	$P<0·005$	30
CH50	C3	0·61	$P<0·005$	47
CH50	C4	0·34	$0·010<P<0·025$	47
CH50	C5	0·57	$P<0·005$	31
CH50	C9	0·31	$0·10<P<0·25$	25
CH50	$\bar{B}$	0·31	$0·05<P<0·10$	31
CH50	$\bar{D}$	0·19	$0·25<P<0·50$	29
CH50	C(3–9)H50	0·41	$0·025<P<0·05$	24
CH50	CV50	0·44	$0·010<P<0·025$	32
AP50	C3	0·41	$0·025<P<0·05$	29
AP50	C4	0·22	$0·10<P<0·25$	29
AP50	$\bar{B}$	0·73	$P<0·005$	30
AP50	$\bar{D}$	0·39	$0·025<P<0·05$	28
AP50	C5	0·34	$0·05<P<0·10$	31
AP50	C9	0·08	$0·50<P$	25
AP50	C(3–9)H50	0·03	$0·50<P$	24
AP50	CV50	0·58	$P<0·005$	32
CV50	$\bar{B}$	0·45	$0·025<P<0·05$	20
CV50	$\bar{D}$	0·58	$P<0·005$	28
C(3–9)H50	C5	0·07	$0·50<P$	22
C(3–9)H50	C9	0·01	$0·50<P$	16

r = Correlation coefficient; P = probability; n = number of persons.

expressed as CH50. Haemolysis of RRBC by components of the alternative pathway has been reported by Platts-Mills & Ishizaka (1974). We reported that this phenomenon could be used as an indicator of the activity of the alternative pathway. Since a straight line was obtained between the dilution of serum and y/(1-y), it was possible to obtain a dilution of the serum to give 50% haemolysis, which was tentatively called AP50 (Takada & Takada, 1978). The alternative pathway was composed of the early part (IF, P, B, D, C3b) and the late part (C3–C9), which is common with the classical pathway. Changes in AP50 may primarily represent changes in the late part of the pathway.

Cobra venom factor (CVF) has been known to activate the alternative pathway (Flexner & Noguchi, 1903; Ballow & Cochrane, 1969). CVF was shown to form a complex with C3 proactivator (B), which functioned as C3 convertase (Müller-Eberhard & Fjellstrom, 1971; Hunsicker *et al.*, 1973). From this, AP50 should be correlated with $\bar{B}$ and CV50. As shown in Table 1, there was a good correlation between AP50 and $\bar{B}$ ($r = 0·73$), and AP50 and CV50 ($r = 0·58$), respectively. On the other hand, AP50 did not have any correlation with C(3–9)H50 or C9. C(3–9)H50 was measured by adding EAC142 (C3 convertase) to EDTA-treated serum, thus haemolysis depended upon amounts of C3–C9 in the serum. Since AP50 did not change in accordance with changes in C3–C9, it could be said to represent primarily the early components of the alternative pathway.

It is also of interest that CH50 had a good correlation with AP50, and that CH50 has some correlation with $\bar{B}$. Although the correlation between AP50 and CH50 may be explained by the fact that both pathways share C3–C9, there may be an interaction among the early components of both pathways since AP50 is not greatly influenced by changes in C(3–9)H50, in contrast to CH50. As CH50 has a correlation with $\bar{B}$, it is possible that B is somehow related to the early parts of the classical pathway. This possibility is currently being pursued.

Finally, it is of interest that C(3–9)H50 has little to do with changes in C5 and C9. Recently, Mayer (1977) proposed a 'doughnut model' for the mechanism of lesion caused by complement. The hypothesis presented by Mayer is that the primary lesion is a transmembrane channel that is formed by the insertion

of hydrophobic peptides from C5b, C6, C7, C8 and C9. Since C5–C9 work as a unit, change in one component of C5–C9 may not influence C(3–9)H50.

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