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The Role of Cellular Antigens in Complement-induced Cytocidal, Immune Adherence, and Phagocytic Reactions

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SUMMARY

The role of antigen, antibody, and specific complement components in cytocidal, immune adherence, and phagocytic reactions is reviewed briefly. Special details on immune adherence are emphasized since antigens which are either soluble or particulate may be detected with striking sensitivity provided that the antigen-antibody complex will interact with complement. Antigens on cell surfaces which are located at sites which are not susceptible to a cidal or lytic effect may be detected either by immune adherence or by phagocytosis. A method for performing quantitative phagocytic reactions is reviewed briefly. Estimations have been made of the molecular requirements of antibody and complement components for these reactions.

INTRODUCTION

Immune adherence was first described in late 1949 as a general phenomenon whereby any antigen-antibody complex which reacts with complement (C′) will then combine with a specific protein receptor site on primate erythrocytes or nonprimate platelets (16). Since then, experimental evidence has been published for a clear-cut differentiation of immune-adherence from "acid-adhesion" and "complement-dependent mixed agglutination" (14) and for the necessity of only the first four components of C′ for immune adherence (26), as well as for phagocytosis (6, 18). In addition, van Loghem and van der Hart (30) have demonstrated that the immune adherence receptor site is present on both primate and nonprimate leukocytes. A technically simple method for measuring immune adherence by agglutination of the "indicator" human erythrocytes was employed in late 1955 using zymosan particles or starch granules, as shown in a photomicrograph published elsewhere (19). This was adapted to a variety of other antigens, including poliovirus (19) and soluble antigens (23).

A comprehensive review on immune adherence was published in 1963 (15). The present report summarizes additional evidence for certain practical uses of immune adherence and comparative descriptive material on immune adherence and cytotoxic and phagocytic assays; it briefly outlines some preliminary and as yet unpublished results done by members of the staff of the Laboratories of Microbiology of the Howard Hughes Medical Institute, as well as certain pertinent experiments done by other groups on the application of immune adherence to the detection of antigen, antibody, and C′ components on a variety of cell types, including those of malignant tissues in experimental animals.

MATERIALS AND METHODS

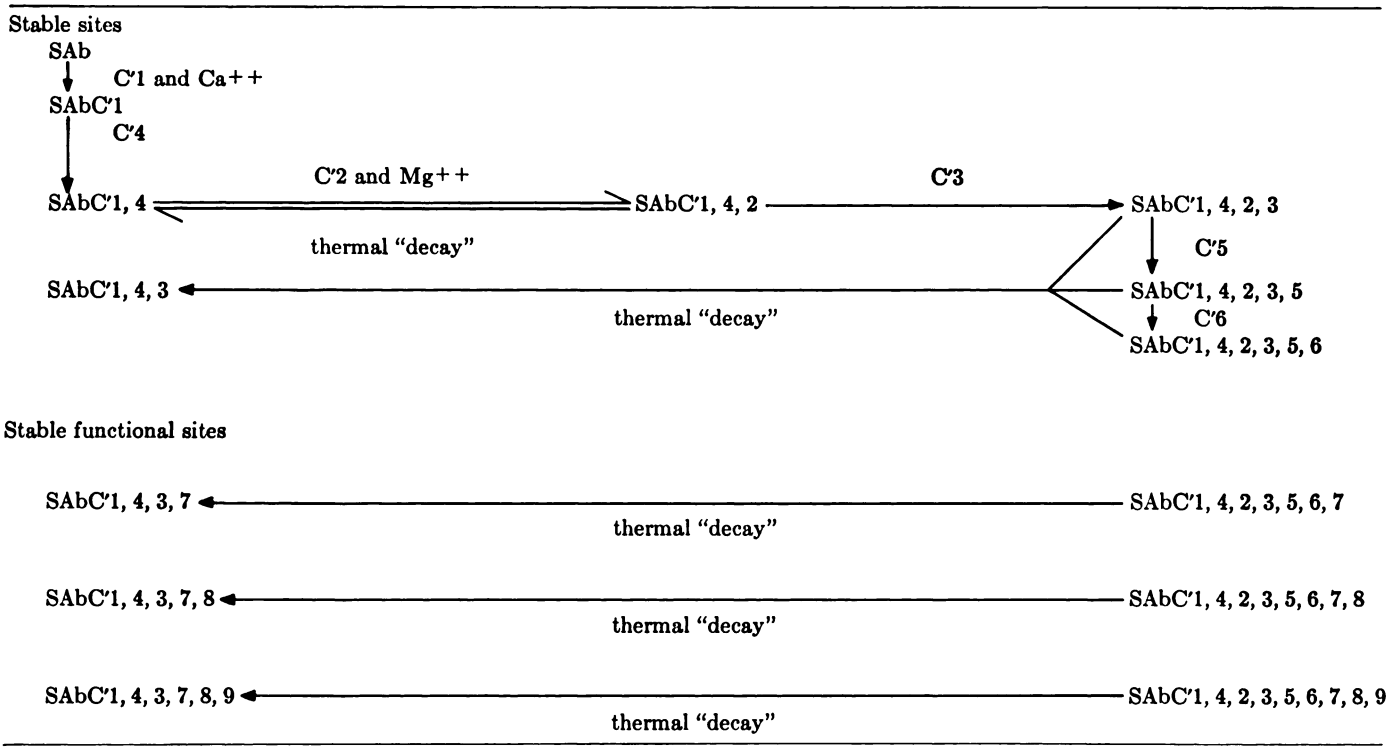
Collection and storage of sheep and human erythrocytes (E), methods for the preparation of partially purified antibody (Ab) and of C′ components and for the generation of the stable EAbC′-component intermediate complexes have been described previously (20, 23). Experiments on "complement-dependent mixed agglutination" were performed by mixing thoroughly various EAbC′-component intermediate complexes with one of several particulate antigens sensitized with their respective antibodies and examining the suspensions microscopically after mixing at 30°C for 30 min.

EXPERIMENTAL OBSERVATIONS AND DISCUSSION

It is now established that nine proteins of the complement system are required for cytocidal or cytolytic reactivities of human, guinea pig, and rabbit serums (20, 21, 23). In contrast, only four components of C′, i.e., C′1, C′4, C′2, and C′3 must react sequentially to produce a site (S) which is reactive in immune adherence and phagocytosis. Once these proteins have reacted with the antibody-sensitized site, both C′1 and C′2 may be removed without loss of reactivity in either of the two immune phenomena. A summary of the stable sites generated by C′ components and of certain biologic activities either of the site or of products released into the fluid phase during generation of the site are shown in Tables 1 and 2 respectively. Since the results on C′1-dependent mixed agglutination have not been published, it is necessary to mention some details on this phenomenon. Sensitized starch granules, zymosan particles, *Salmonella typhi*, as well as a washed specific precipitate of bovine serum albumin-antialbumin, were washed thoroughly and mixed with sheep E, sheep EAb, and with the various sheep EAbC′ intermediate complexes. Coagglutination resulted only with the intermediate complexes possessing C′1 sites. In every case the coagglutination was blocked in the presence of ethylenediamine tetraacetate and, in fact, the agglutinated complex could be dissociated by treatment with the same chelating agent.

Of equal interest, especially from the standpoint of control mechanisms, three inactivators of C′ components have been found in normal serum of human beings, guinea pigs, and

Table 1



Pathways leading to the formation of stable complexes of complement components and the antibody site on cell membranes.

Table 2

SAbC'1	1. Site is reactive in "complement-dependent mixed agglutination."
	2. Site possesses esterase activity.
SAbC'4, 3	1. Site is reactive in immune adherence.
	2. Site is reactive in phagocytosis.
SAbC'1, 4, 2, 3	1. Chemotactic fragment is released during generation of this site (32).
	2. Anaphylatoxin fragment is released during generation of this site (4).
SAbC'1, 4, 2, 3, 5	1. Anaphylatoxin fragment is released during generation of this site (7).
SAbC'1, 4, 2, 3, 5, 6, 7	1. Chemotactic fragment is released during generation of this site (32).

Some biologic properties of sites sensitized with components and of fragments produced during generation of the sensitized site. At 0°C hemolytically active C'1 will dissociate from a site sensitized with IgM Ab.

rabbits[1] (22, 28). The potential importance of these inactivators *in vivo* has been discussed briefly elsewhere (22).

Now that nine components and three inactivators of the complement system have been isolated in a functionally pure state and several have been shown to meet certain of the criteria for chemical purity, it has been possible to begin to define the amounts of these proteins in normals (21) and in individuals with different disease syndromes. It has long been known that total complement reactivity may be elevated or depressed in certain diseases, but usually this led to little or no insight into the mechanisms of the disease, since the component or inactivator responsible could not be identified. Using purified components in our laboratory, Drs. McKensie and Colsky of the University of Miami have made initial studies of specific component activities in patients with various types of malignancy. Their results will be published shortly in *Cancer Research*. In addition, the measurement of total complement and of individual components has given insight into the possible mechanism of a normal state of resistance to bacterial and viral infection in members of a family known to possess a deficiency of C'2 (8). As shown in Table 3, serum from one individual showed a marked deficiency of C'2, as measured either by hemolysis or by immune adherence. Nonetheless, the

[1] Gigli, I., and Nelson, R. A. Naturally-occurring Inhibitors of C'3 and C'6 in Human Serum, manuscript in preparation.

Table 3

Serum	Total C′ titers		Hemolytic titers of C′ components[a]									C′2 titer by I. A.
	Hemolytic	I. A.	1	4	2	3	5	6	7	8	9	
Control	192	800	60,000	200,000	7,800	1,600	3,200	100,000	3,200	100,000	150,000	100,000
Deficient	10	600	60,000	200,000	<10	1,600	3,200	50,000	3,200	100,000	150,000	600

Measurements of C′ and C′ components in serum from an individual with a genetic deficiency of C′2. (Unpublished experiments by Drs. David Vroon and R. A. Nelson done on serum generously donated by Dr. Harold C. Woodworth of the Communicable Disease Center, Chamblee, Georgia.) I. A., measurements of immune adherence using human erythrocytes as indicator particles.

[a] Hemolytic titers are only approximate since they usually were performed in "microtiter assay" and with purified guinea pig components as reagents. In more recent experiments by Vroon using purified human components, the titers of C′3, C′5, and C′6 have been found to be between 10 and 20 times higher than those recorded here.

total C′ reactivity in immune adherence was almost the same as that of a control serum. Indeed it is noteworthy that C′2 as measured by immune adherence was the same as total C′ reactivity measured by the same method. Apparently, this amount of C′2 is adequate to permit immune adherence and presumably permit phagocytosis to proceed *in vivo* at a rate which is adequate to afford a normal state of resistance to infectious agents, as theorized in earlier studies on immune adherence (17).

Quantitative methods for measuring Ab and C′ components by hemolytic assays have been developed in several laboratories. Taverne (29) was the first investigator to apply quantitative technics in immune adherence using radio-labeled T2 bacteriophage. Nishioka further demonstrated the usefulness of quantitative methods using crystalline bovine serum albumin labeled with ^{131}I as antigen (25). As yet, the use of labeled Ab in immune adherence systems has not been reported. Supposedly quantitative methods for measuring phagocytosis have been reported by many investigators. However, they all suffer from the limitation that they failed to differentiate between antigen which had been ingested and antigen which was attached to the surface of the phagocytic cell by immune adherence. The experiments originally reported on the requirement of C′1, C′4, C′2, and C′3 for phagocytosis using sensitized sheep erythrocytes as the particulate antigen have been extended by Gigli and Nelson to quantitate both C′ components and Ab[2] (6).

Of equal interest, it has been possible to demonstrate antibody on the cells of homografted individuals (5) and on malignant cells from mice injected with Ehrlich ascites cells, as well as on cells from "spontaneous" breast tumors in inbred mice (1). All three of these lines of experimentation employed immune adherence to detect so-called "cell-bound" antibody. The rationale behind the use of immune adherence was based on the extreme sensitivity of this method and on the fact that many types of malignant cells are difficult or impossible to lyse even in the presence of "excess" C′. Table 4 shows the comparative sensitivity of the three-assay methods for the detection of antibody, as well as an estimate of the amounts of C′ components required for hemolysis and immune adherence. From these data and from previously published results (18), it may be estimated that in certain systems immune adherence may detect as little as 0.008 μg Ab nitrogen per ml of serum and that in our routine assays about two molecules

[2] Gigli, I., and Nelson, R. A. Molecular Requirements for Human and Rabbit Antibody in Immune Phagocytosis, manuscript in preparation.

Table 4

Serum	Protein	Approx. μg protein per ml serum	Approx. maximal no. of molecules for 50% end point		
			Hemolysis	Immune adherence	Phagocytosis
Immune	IgG	576.6	2,000	1,100	11,100
	IgM	86.8	58	2	110
Normal	C′1	150	10	2	ND
	C′4	300	100	20	ND
	C′2	250	380	76	ND
	C′3	240	150	15	ND
	C′5	100	45	NR	ND
	C′6	140	130	NR	ND
	C′7	90	100	NR	ND
	C′8	50	10	NR	ND
	C′9	10	12	NR	ND

Estimations of the content of IgG and IgM Ab in a rabbit antiserum and of C′ components in normal guinea pig serum and of the maximal number of molecules per erythrocyte required for hemolysis and immune adherence. Partially purified antibodies were prepared from serum of an individual rabbit immunized for about 30 days with about 2.3×10^{10} washed sheep erythrocytes. NR, not required; ND, not done.

of IgM Ab and 10 to 15 molecules of C'3 per erythrocyte are adequate to induce easily detectable hemagglutination patterns with human erythrocytes as the indicator particle. More recent data[2] indicate that under optimal circumstances similar sensitivity may be achieved using quantitative measurements of phagocytosis. The striking discrepancy between our estimates of the number of molecules of C'3 required for hemolysis and immune adherence and those values described by other investigators is discussed elsewhere (21).

Although there is not adequate time to go into detail, there are two examples of Ab and C' induced phenomena using tumor cells which may be of general interest to members of this Society. Based upon the well-known change in pH of the fluid phase as cells metabolize *in vitro*, Stolfi, in our laboratory, has employed a method for detecting Ab on Ehrlich ascites cells suspended in a conventional tissue culture medium containing phenol red and mixed with dilutions of Ab made in "microtiter plates" (27). While not yet developed as a quantitative measurement, the method is extremely useful for screening large numbers of samples of homologous or heterologous antisera for their ability to inhibit metabolism of this particular type of celi. Another promising line of investigation on mechanisms of resistance to tumor cells has been started, again using the Ehrlich ascites cells. As is also well known, heterologous Ab will prevent growth of these malignant cells when mixed *in vitro* before injection into mice. When these Ab-sensitized cells were treated with any combination of the nine highly purified components of guinea pig C', similar "protection" resulted. However, when treated with C'1 through C'7 and then with a C'8 preparation which had lost its lytic effect but which still possessed combining sites, the "protective effect of the Ab was to a large extent lost, presumably because the C'9 of the mice was unable to lyse the malignant cells. Although much remains to be studied using such methods, it is of considerable interest to note that the treatment of guinea pigs with a single injection of a highly purified "inactivator" of C'3 found in cobra venom will completely protect them against the intravenous injection of between two to five 95%-lethal doses of Forssman antibody. Similarly, this "inactivator" of C'3, discovered and purified in our laboratory in 1964, has subsequently been shown to produce a striking delay in the rejection of renal heterografts in dogs.

Finally, it should be mentioned that immune adherence has found practical application in several immune systems for the detection of either antigen or antibody. Some of these include experiments with *Treponema pallidum* (3, 13), Leptospira (11), tubercle bacilli (9), staphylococci (10), leukemic cells (2), lymphocytes or fibroblasts from skin (12, 31); J. J. van Loghem, unpublished material presented at the National Institute of Allergy and Infectious Disease Meeting of the Transplantation Immunology Branch, October 2, 1967), bacteriophage (29), poliovirus (19), and several other soluble antigens (24).

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