Immune Adherence by the Alternate Complement Pathway (36760)

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Immune adherence (IA) is the term used to describe a specific immunologic reaction wherein microorganisms or other antigens sensitized with antibody and complement become attached to the surface of untreated primate erythrocytes or nonprimate platelets (the indicator particles). The occurrence of IA *in vivo* has been previously reported (1), and several investigators have speculated on its possible role in the promotion of phagocytosis, resistance to infection, and the generalized Schwartzman reaction (2). Because of the extreme sensitivity of the IA reaction, it has been widely used in the laboratory as an *in vitro* test for the detection of antigen, antibody, and bound complement components. Nishioka and Linscott (3) first showed that the generation of an IA reactive complex required the specific binding of only the first four components of complement (C1, C4, C2, and C3), and further studies have established bound C3 as the primary component capable of mediating IA (4–6).

Recent studies have indicated the existence of at least 2 mechanisms for activation of the late components of complement (C3–9) in serum with generation of biologically active complement fragments (7–10)—the classical mechanism involving the sequential action of C1, C4, and C2, and the alternate or bypass pathway involving the C3 proactivator, properdin, and other components yet to be delineated. The biologic role of this latter pathway remains to be defined. The experiments reported here demonstrate the ability of immune complexes and endotoxin to produce IA via the alternate complement pathway, and the inability of an antibody directed to an intact mammalian cell membrane to generate this reaction by the bypass mechanism.

Materials and Methods. For the study of alternate pathway generated IA, serum from guinea pigs with a total genetic deficiency of C4 (C4D) (11) was used as a source of the components of the alternate pathway free of activity of the classical pathway. Serum from NIH "multipurpose" guinea pigs, the strain from which the C4D guinea pigs were derived, was used as the normal serum control, and cobra venom factor (CVF)-treated guinea pig serum was used as a source of serum exhausted of C3–9. Serum with a C3–9 titer of less than 1% of normal was obtained from normal guinea pigs 20 hours after they had received an intraperitoneal injection of 20 units/100 g body weight purified cobra venom factor (lot No. 40021, Cordis Labs, Miami, FL).

Immune adherence tests were performed in microtiter plates (Cooke Engineering). To 25 μl of serially diluted normal, C4D or CVF-treated guinea pig sera, an equivalent volume of either 2-fold falling dilutions of endotoxin (*E. coli* 0111:B4, lot No. 564550, Difco Laboratory, Detroit, MI), immune complexes of BSA-rabbit anti-BSA [prepared as described in Ref. (10)] or complexes of sheep erythrocyte stromata–rabbit anti-Forssman antibody were added. In the latter case, dilutions of anti-Forssman antiserum containing mostly IgM antibody and stroma were chosen to produce the equivalent of lightly to optimally sensitized sheep erythrocytes (EA). After these mixtures were shaken for 15 min at 37°, 50 μl of guinea pig platelets, prepared by the method of Siqueira and Nelson (12) were added. The plates were incubated with shaking at 37° for 60 min, and the degree of platelet agglutination

Immune complexes

	Serum dilutions →											
Immune complexes	μg/ml	1/1	2	4	8	16	32	64	128	256	512	1024	VBS
Normal serum ↓	100	Tr	2+	3+	3+	3+	2+	1+	1+	2+	Tr	—	—
	50	1+	2+	3+	3+	2+	2+	2+	—	Tr	—	—	—
	25	1+	2+	3+	3+	2+	2+	2+	—	—	—	—	—
	12	Tr	2+	3+	3+	Tr	Tr	—	—	—	—	—	—
	6	Tr	2+	3+	1+	—	—	—	—	—	—	—	—
	3	Tr	Tr	1+	Tr	—	—	—	—	—	—	—	—
	1.5	Tr	Tr	1+	1+	—	—	—	—	—	—	—	—
	VBS	Tr	Tr	1+	Tr	—	—	—	—	—	—	—	—
C4D serum	100	1+	2+	3+	3+	2+	1+	1+	1+	—	—	—	—
	50	1+	3+	2+	2+	1+	—	—	—	—	—	—	—
	25	1+	2+	3+	2+	1+	Tr	—	—	—	—	—	—
	12	1+	2+	1+	1+	—	—	—	—	—	—	—	—
	6	Tr	2+	1+	Tr	—	—	—	—	—	—	—	—
	3	Tr	1+	1+	Tr	—	—	—	—	—	—	—	—
	1.5	Tr	2+	2+	Tr	—	—	—	—	—	—	—	—
	VBS	Tr	1+	Tr	—	—	—	—	—	—	—	—	—

Endotoxin

	Serum dilutions →											
Endotoxin	μg/ml	1/1	2	4	8	16	32	64	128	256	512	1024	VBS
Normal serum ↓	500	2+	2+	3+	2+	3+	Tr	—	—	—	—	—	—
	250	2+	2+	3+	3+	3+	1+	—	—	—	—	—	—
	125	2+	2+	2+	3+	2+	—	—	—	—	—	—	—
	60	1+	2+	3+	3+	3+	Tr	Tr	Tr	—	—	—	—
	30	1+	2+	3+	3+	1+	—	—	—	—	—	—	—
	15	1+	1+	3+	2+	Tr	—	—	—	—	—	—	—
	75	1+	2+	3+	2+	1+	—	—	—	—	—	—	—
	VBS	1+	2+	1+	—	—	—	—	—	—	—	—	—
C4D serum	500	Tr	1+	3+	3+	3+	1+	—	—	—	—	—	—
	250	1+	2+	3+	1+	1+	—	—	—	—	—	—	—
	125	1+	2+	2+	2+	1+	—	—	—	—	—	—	—
	60	Tr	1+	2+	2+	Tr	—	—	—	—	—	—	—
	30	Tr	1+	2+	1+	1+	Tr	—	—	—	—	—	—
	15	Tr	2+	2+	1+	—	—	—	—	—	—	—	—
	75	Tr	2+	2+	—	—	—	—	—	—	—	—	—
	VBS	Tr	2+	1+	—	—	—	—	—	—	—	—	—

FIG. 1. Generation of immune adherence by immune complexes and endotoxin in normal and C4D guinea pig serum. VBS = Veronal buffered saline; Tr = trace.

was then immediately determined.

Results and Discussion. Typical results illustrated in Fig. 1 show that IA could be generated both in normal and C4D sera by immune complexes and endotoxin. The results could be duplicated with C4D serum in

Sensitized sheep erythrocyte stromata

		Serum dilutions →											
Sensitized stromata		1/1	2	4	8	16	32	64	128	256	512	1024	VBS
Normal Serum ↓	1/1	Tr	2+	2+	4+	4+	4+	3+	—	—	—	—	—
	2	Tr	1+	2+	2+	3+	3+	3+	—	—	—	—	—
	4	Tr	Tr	1+	2+	2+	2+	2+	—	—	—	—	—
	8	Tr	Tr	1+	1+	2+	2+	1+	—	—	—	—	—
	16	—	Tr	Tr	2+	2+	2+	1+	—	—	—	—	—
	32	—	Tr	1+	1+	1+	1+	1+	—	—	—	—	—
	64	—	Tr	1+	1+	1+	1+	1+	—	—	—	—	—
	VBS	Tr	Tr	Tr	1+	Tr	1+	—	—	—	—	—	—
C4D serum	1/1	Tr	1+	1+	Tr	—	—	—	—	—	—	—	—
	2	Tr	Tr	—	—	—	—	—	—	—	—	—	—
	4	Tr	Tr	—	—	—	—	—	—	—	—	—	—
	8	Tr	Tr	Tr	Tr	—	—	—	—	—	—	—	—
	16	—	—	—	—	—	—	—	—	—	—	—	—
	32	—	—	—	—	—	—	—	—	—	—	—	—
	64	—	—	—	—	—	—	—	—	—	—	—	—
	VBS	—	Tr	Tr	—	—	—	—	—	—	—	—	—

FIG. 2. Generation of immune adherence by sheep erythrocyte stromata sensitized with rabbit anti-Forssman antibody.

the presence of 0.01 *M* ethylene glycol tetraacetic acid (EGTA), indicating a lack of any role for C1 in this reaction. IA was not produced by either immune complexes or endotoxin when CVF-treated sera were used as the complement source, substantiating the central role of the terminal components of complement (C3) in this reaction.

When sheep erythrocyte stromata–rabbit anti-Forssman antibody complexes were allowed to interact with normal and C4D sera, IA was generated in only the normal serum (Fig. 2). This is in agreement with recent studies demonstrating in a number of different intact cell membrane–antibody systems, that antibodies fixed to intact mammalian cell membrane antigens were incapable of activating the alternate pathway. The only exception noted was in the Forssman system when very large amounts of sensitizing antibody were used (13). With large amounts of antibody, the late components of complement could be activated by a complex mechanism to be discussed in detail elsewhere.

It is known that the alternate pathway has the capacity to mediate bactericidal and phagocytic reactions (14), and can be activated by endotoxin and soluble antigen–antibody complexes with generation of biologic products with phlogistic activity (15, 16). Since IA has been implicated in many *in vivo* host defense functions such as clearance of endotoxin from the circulation and production of thrombocytopenia following endotoxin administration, it is of importance to determine whether this mechanism can be activated by the two major complement pathways. The findings here that IA is adequately generated by the bypass mechanism would further support a major role of this pathway in inflammatory reactions and host defense.

Summary. The ability of the alternate complement pathway to generate the immune adherence reaction was studied in C4D guinea pig sera. It was found that IA via the alternate pathway could be generated by endotoxins and immune complexes, but that this phenomenon could not be produced by optimally sensitized intact mammalian cell membranes.

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