

Human Lymphocytes Bear Membrane Receptors for C3b and C3d

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ABSTRACT Human peripheral blood lymphocytes have membrane receptors for EAC43b (sheep erythrocytes sensitized with antibody and complement) and also for EAC43d, obtained by treating EAC43b with C3b inactivator. Human granulocytes bind only EAC43b. C3 fragments obtained by limited trypsin digestion of purified human C3 display both C3b and C3d sites, since they inhibit rosette formation of lymphocytes with EAC43b and EAC43d. These findings raise the possibility that C3b and C3d receptor sites may be selectively distributed among normal subpopulations of B lymphocytes as well as among leukemic leukocytes.

INTRODUCTION

B lymphocytes, as well as granulocytes, monocytes, and macrophages from many mammalian species, bind sheep erythrocytes (E),¹ sensitized with antibody (A) and complement (C) to form rosettes (1). The binding is through a receptor for modified C3 on the membrane of the reacting cell. Recently, it has been observed that while human peripheral blood B lymphocytes form rosettes with EAC43_{human} (prepared with purified human C components) and with EAC_{mouse} (prepared with whole mouse serum as a source of C), human granulocytes react only with EAC43_{human} (2). One factor that could account for this differential reactivity could be the nature of the C3 fragments bound to the two kinds of sensitized erythrocytes. EAC43 prepared with purified C components bear C3b on the membrane. It is known that serum contains an enzyme, C3b inactivator, or conglutinogen-activating factor (3-5), which splits membrane-bound C3b into two fragments: C3c, which is released into the medium, and C3d, which remains associated with the cell surface. Erythrocytes bearing C3b or C3d have different properties: EAC43b but not

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¹*Abbreviations used in this paper: A, antibody; C, complement; E, Sheep erythrocytes; EDTA-GVB, ethylenediamine tetraacetic acid-gelatin Veronal-buffered saline.*

EAC43d bind to human erythrocytes (classical immune adherence reaction) and only EAC43d is agglutinated by conglutinin. We show here that human B lymphocytes have membrane binding sites for both EAC43b and EAC43d while granulocytes preferentially bind EAC43b.

METHODS

Human lymphocytes were obtained from peripheral blood (2) and from tonsils. Human granulocytes were obtained from the leukocyte-rich plasma of dextran-sedimented heparinized blood. This preparation usually contained about 15% of mononuclear cells. The leukocytes were suspended in phosphate-buffered saline, pH 7.4. The sources and preparation of E, EA, and EAC_{mouse}, have been previously described (1). Functionally purified complement components and human C3b inactivator were purchased from Cordis Corp., Miami, Fla. EDTA-GVB (ethylenediamine tetraacetic acid-gelatin veronal-buffered saline) was prepared according to the method of Rapp and Borsos (6). EAC_{4human} cells were prepared with purified guinea pig C1 and human EDTA-serum by the method given in reference 7. EAC43_{human} were prepared by adding purified guinea pig C1 and human C2 and C3 to EAC_{4human} according to Rapp and Borsos' method (6). C3b, obtained by limited trypsin digestion of purified human C3, was a gift from Dr. V. Bokisch. EDTA-serum used as a source of C3b inactivator was 0.01 M with respect to EDTA. The technique for rosette formation has been described in detail (1). Immune adherence between the various EAC and type O human red cells was performed as described by Lachmann and Müller-Eberhard (4).

RESULTS

The EAC43_{human} cells, presumably having C3b on their membranes, were incubated with various dilutions of human EDTA-serum for 40 min to generate the EAC43d intermediate. Then the red cells were washed and tested for rosette formation with granulocytes and lymphocytes, and for immune adherence with human erythrocytes (Table I). As expected, after EDTA-serum treatment the EACs were negative in immune adherence. The proportion of granulocytes which formed rosettes with EAC4, EAC43b, and EAC43d was 9.8, 44.0, and 8.0%, respectively, while there was no significant difference in the number of rosettes formed between

TABLE I
Effect on Rosette Formation and Immune Adherence of Treating EAC43* with C3b Inactivator

Type of leukocyte	Indicator cells	Dilution of EDTA-serum (or purified C3b inactivator) used to treat indicator cells	Rosettes‡	Immune adherence between human type O erythrocytes and indicator cells
			%	
Granulocytes	EA	No treatment§	1.4	—
	EAC4	No treatment	9.8	—
	EAC43	No treatment	44.0 (27.0)	+
	EAC43	1/64	19.3	±
	EAC43	1/32	8.3	—
	EAC43	1/16	8.0	—
	EAC43	1/8	7.4 (4.0)	—
Blood lymphocytes	EAC4	No treatment	2.3	—
	EAC43	No treatment	11.6 (13.1)	+
	EAC43	1/4	11.9 (15.8)	—
Tonsil lymphocytes	EAC4	No treatment	1.2	—
	EAC43	No treatment	37.4 (32.0)	+
	EAC43	1/4	38.8 (27.8)	—

* EAC43 were prepared with purified human C components.

‡ The numbers in parenthesis refer to results obtained with EAC43_{human} treated with a purified preparation of C3b inactivator (initial concentration: 500 U/ml).

§ The cell intermediates were incubated in EDTA-GVB.

EAC43b or EAC43d and lymphocytes. Similar results were obtained when EAC43b were incubated with a purified preparation of C3b inactivator instead of EDTA-serum, and when the subsequent incubations with the leukocytes were performed in the presence or in the absence of 0.01 M EDTA.

The effect of EDTA-serum on preformed rosettes is shown in Table II. Here EDTA-serum was added to cell suspensions containing granulocyte- or lymphocyte-EAC43_{human} rosettes and the tubes were incubated at 37°C. At various intervals, samples were taken and the number of remaining rosettes, as well as the total number of leukocytes, was counted. The number of rosettes in the granulocyte-EAC43-EDTA-serum mixtures was

greatly decreased compared with serum-free control mixtures. The decrease was due to dissociation of rosettes rather than to nonspecific cell losses or cell death, since the total number of viable cells present remained constant throughout this period. However, the number of rosettes in the lymphocyte-EAC43-EDTA-serum mixture was not decreased compared with control mixtures without serum.

To gain further insight into the specificity of the C3 sites on the leukocyte membrane, the effect on rosette formation of adding purified C3b fragments to the incubation medium was studied. In several experiments, one of which is shown in Table III, it was found that C3b inhibits rosette formation by granulocytes and lymphocytes with both EAC43b and EAC43d.

TABLE II
Dissociation by Treatment with EDTA-Serum of Rosettes Formed between EAC43b and Granulocytes

Time	% rosettes with granulocytes remaining after incubation in		% rosettes with peripheral blood lymphocytes remaining after incubation in	
	EDTA-serum	EDTA-GVB	EDTA-serum	EDTA-GVB
<i>min</i>				
0	46.0	46.0	12.9	12.9
30	21.9	41.5	8.8	10.1
60	10.5	35.8	7.0	7.4

DISCUSSION

The experiments described furnish evidence that the C3 receptors on B cells and on granulocytes do not have identical specificities. B lymphocytes bind EAC43b as well as EAC43d. That is, they may either have a membrane receptor for the C3d moiety contained in C3b or they may have two separate receptors for different sites on the C3b molecules. Some observations suggest the existence of more than one C3 receptor on normal human lymphocytes. Leukemic B cells from some patients with chronic lymphocytic leukemia that react well with

TABLE III
Inhibition by C3b of Rosette Formation Between Leukocytes
and EAC43b* or EAC43d†

Leukocytes	Indicator cells	Final concentration of C3b in the incubation medium		Rosettes
		$\mu\text{g/ml}$	%	
Peripheral blood lymphocytes	EAC43b	0	10.5	
		40	2.2	
Tonsil lymphocytes	EAC43d	0	7.4	
		40	2.2	
		160	0.8	
	EAC43b	0	32.9	
		40	2.3	
		160	23.0	
Granulocytes	EAC43d	0	6.9	
		40	0.6	
		160	88.4	
	EAC43b	0	66.4	
		16	52.1	
		40	8.9	

* EAC43b were prepared with purified human C components.

† EAC43d were prepared by treating the EAC43b with a preparation of purified human C3b inactivator for 1 h at 37°C. The EAC43d cells were immune adherence negative.

EAC_{mouse} react poorly or not at all with EAC_{human} (8). Also, certain human lymphoid cell lines do not react with EAC43 cells made with partially purified mouse C3. However, they do bind EAC43_{mouse} after treatment of the erythrocytes with a mouse serum fraction which presumably contains mouse C3b inactivator since after such treatment the EAC43_{mouse} become immune adherence negative (9). These findings suggest that normal human B lymphocytes have two receptors for membrane-bound C3, one for C3b, and another for C3d, and that the leukemic cells from some patients have either lost or never possessed C3b receptors.

The observation that C3b inhibits the interaction of both types of receptors with their substrates implies that this fragment displays both the C3b and the C3d sites. However, the possibility that the preparation contains some further degradation products of C3b cannot be excluded.

In contrast to B lymphocytes and leukemic cells, only receptors for EAC43b are readily demonstrated on human granulocytes. These findings provide the explanation for the observation that EAC43 prepared with guinea pig components are not phagocytosed by guinea pig granulocytes after treatment with partially purified C3b inactivator (10). It should be pointed out that receptors for C3d may also be present on these cells, but their distribution, low number, or low affinity may not be conducive to binding of large particles such as sheep erythrocytes. The few rosettes observed between EAC-

43d and granulocytes (Table I) might be the result of such interaction. Alternatively, these clusters may have formed on some mononuclear cells contaminating these preparations of granulocytes, or simply result from EAC4 binding.

C3 receptors are probably of importance in phagocytosis (10-13), in the triggering of the release of vasoactive amines from platelets (14), and perhaps, as recently suggested, in the initiation of the immune response (15). The existence of separate membrane receptors for different C3 split products, as well as their selective distribution on normal and leukemic leukocytes, raises new questions as to their respective effector functions in vivo.

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