Agents and Actions
vol. 6 · 1/2/3 (1976)
Birkhäuser Verlag, Basel

Opsonizing Activities of IgG, IgM Antibodies and the C3b Inactivator-Cleaved Third Component of Complement in Macrophage Phagocytosis

B. Wellek, H. Hahn and W. Opferkuch
Institut für Medizinische Mikrobiologie, Johannes Gutenberg-Universität, 65 Mainz, Augustusplatz, und Lehrstuhl für Medizinische Mikrobiologie, Ruhruniversität Bochum, 463 Bochum

Abstract

Phagocytosis of SRBC by guinea-pig peritoneal macrophages is enhanced by opsonizing IgG antibody alone. IgM antibody requires the presence of bound C3. Treatment of C3b coated SRBC with purified C3b inactivator (yielding EA_{IgM} C1423d) does not reduce attachment to, and phagocytosis by, peritoneal macrophages. This finding suggests the existence of a C3d receptor on peritoneal macrophages. EC43b intermediates which have been produced by removing IgM antibody by mercaptoethanol treatment and by subsequent removal of C1 and C2, are phagocytosed despite the absence of IgM antibody. Furthermore, treatment of EC43b with C3b inactivator does not change phagocytosis. Thus, IgM antibody does not appear to be a necessary prerequisite for the stimulation of phagocytosis, C3b or C3d alone being sufficient.

Results and discussion

Phagocytosis of particles by macrophages is aided by opsonins such as IgG and IgM antibodies, and the bound third component of complement (C3b) [1–3]. In a detailed study [4] we have found that IgG antibody bound to SRBC at optimal concentrations very effectively opsonizes by itself, a finding which has previously also been reported by others [1]. C3b visibly enhances the uptake of IgG-coated particles only at limiting concentrations of IgG antibody [2, 4]. On the other hand, IgM coated particles at all concentrations of IgM require the presence of C3b in order to stimulate attachment and phagocytosis [3, 4]. Thus, the bound third component of complement, C3b, is recognized by receptors on the surface of macrophages and, as has been shown by Gigli and Nelson [5], by polymorphonuclear leukocytes.

A C3b inactivator has been found in the sera of several species [6, 7]. This enzyme splits C3b, generating two fragments, C3c and C3d, the latter remaining in the cell-bound state [6]. Receptors for C3d have recently been described to occur on lymphoid cell lines [8, 9]. This observation prompted us to seek evidence whether such receptors play a role in the interaction of phagocytic cells with C3d coated particles. We were able to demonstrate that guinea-pig peritoneal macrophages bind and ingest EA_{IgM} C1423d, i.e. C3b inactivator treated red cell intermediates [10]. On the other hand, in confirmation of Gigli and Nelson's data [5], polymorphonuclear leukocytes are inactive. Thus, peritoneal macrophages, but not polymorphonuclear leukocytes, possess membrane receptors for C3d, mediating both attachment and phagocytosis of C3d coated particles. These receptors ensure attachment and phagocytosis under conditions of C3b inactivator activity.

The relevance of the third component of complement in phagocyte-particle interaction thus having been established, we next intended to investigate whether, in C3-mediated attachment and phagocytosis, IgM antibody is an indispensable component, or whether C3 alone is sufficient to trigger phagocytosis. A method for removing IgM antibody from EA_{IgM} intermediates using mercaptoethanol treatment has been described by Borsos and Leonard [11]. We used this method to produce EC43b and EC43d intermediates. These were phagocytosed by guinea-pig peritoneal macrophages despite the demonstrable absence of IgM antibody, suggesting that C3b or C3d alone can mediate attachment and trigger phagocytosis. Opsonized SRBC were prepared with purified IgM anti-Forssman antibody (immunoelectrophoretically

devoid of IgG) and by the stepwise addition of partially purified guinea-pig C1, C4, C2 and C3 [4, 10]. The resulting intermediate, EA_{IgM} C1423 was treated with VBS buffer containing 0.02 m EDTA in order to remove C1 (30 min, 30 °C). For the removal of IgM antibody, the intermediates were next incubated (60 min, 37 °C) in VBS buffer containing 0.2 m mercaptoethanol. C2 decayed during this procedure, thus leaving EC43d intermediates. EC43d were then subjected to treatment (60 min, 37 °C) with partially purified C3b inactivator to produce EC43d. Absence of IgM antibody was verified by the absence of lytic activity of EC43 intermediates after incubation with 1:40 diluted guinea-pig serum (60 min, 37 °C). Absence of C1 and C2 was confirmed by hemolytic tests (Wellek, Hahn, Opferkuch, manuscript submitted). EC43b were hemolytically active after restoration of the components which had previously been removed; treatment with C3b inactivator (60 min, 37 °C) caused loss of hemolytic C3 activity.

Guinea-pig macrophage monolayers were prepared on coverslips in Leighton tubes using 2×10^6 viable cells from starch gel induced peritoneal exudates [10]. To these monolayers were added either 1.3×10^7 EC43b or EC43d. Phagocytosis was allowed to proceed for 1 hour on a rocking platform at 37 °C in 5% CO_2. Extracellular SRBC were then lysed with Tris-ammoniumchloride buffer [12], the coverslips Wright-stained, and the number of ingested erythrocytes determined under light microscopy. For rosette formation, 0.12×10^6 viable peritoneal cells were each incubated in 9 mm glass ring chambers to form monolayers of adherent cells. 1.3×10^7 EC43b or EC43d, respectively, were then added and incubated for 10 minutes on a rocking platform. After washing, chambers were sealed with coverslips, inverted, and the numbers of rosettes evaluated under phase contrast microscopy. Adherent cells surrounded by four or more erythrocytes were considered rosettes.

Two things become apparent from the data summarized in the table: First, EC43b was still active in rosette formation and phagocytosis despite the absence of IgM antibody. Second, transformation of EC43b into EC43d by treatment with C3b inactivator did not alter the extent of rosette formation and phagocytosis.

The reported findings indicate that guinea-pig peritoneal macrophages can functionally recognize C3d, whereas polymorphonuclear leukocytes cannot. These observations suggest the existence of C3d receptors on macrophages, facilitating both attachment and phagocytosis of C3d opsonized particles. Furthermore, the interaction between bound C3d and C3d receptors on mononuclear phagocytes appears to take place independently of IgM antibody. Thus, stimulation of the C3d receptor alone suffices to cause both attachment and phagocytosis.

Acknowledgments

Purified guinea-pig C3b inactivator was a gift from Dr. Anne Nicholson, Johns Hopkins University Medical School, Baltimore, Md. The authors thank Mrs. C. Berger for expert technical assistance. These studies were supported by the Deutsche Forschungsgemeinschaft, SFB 107.

Phagocytosis and rosette formation of EC43b and EC43d.

SRBC intermediate	preincubation in[1])	rosettes formed[2])	ingested SRBC intermediate[3])	% lysis of SRBC intermediate[4])
$EC43_{(1/9)}$[5])	VBS-G buffer[6])	165	728	100
	VBS-G + C3b inactivator	151	759	0
$EC43_{(1/27)}$[5])	VBS-G buffer	136	476	91
	VBS-G + C3b inactivator	135	492	0
EC4	VBS-G buffer	2	6	0
	VBS-G + C3b inactivator	1	7	0

[1]) 60 minutes, 2 washings, resuspension to original volume.
[2]) Per 200 macrophages. Means of duplicate readings.
[3]) Per 500 macrophages. Means of duplicate readings.
[4]) Hemolytic C3 test after restoration of previously removed components.
[5]) C3 dilution used for preparation of SRBC intermediate.
[6]) VBS buffer containing 5% glucose.

Abbreviations used in this paper:

Complement nomenclature according to Bull. WHO *39*, 935 (1968).

SRBC = sheep red blood cells

EA_{IgM} = antibody (IgM)-coated erythrocyte

EA_{IgM} C1423d = EA_{IgM} C1423b treated with C3b inactivator

VBS(-G) = veronal buffered saline (with added 5% glucose)

References

[1] M. Rabinovitch, *Studies on the immunoglobulin which stimulates the ingestion of glutaraldehyde-treated red cells attached to macrophages,* J. Immun. *99,* 1115 (1967).

[2] B. Mantovani, M. Rabinovitch and V. Nussenzweig, *Phagocytosis of immune complexes by macrophages. Different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin (IgG),* J. exp. Med. *135,* 780 (1972).

[3] H. Huber and M. Weiner, *Binding of immune complexes to human macrophages: The role of membrane receptor sites,* in: W.H. Wagner and H. Hahn, eds., *Activation of Macrophages,* Excerpta Medica, Amsterdam 1974, p. 54.

[4] B. Wellek, H. Hahn and W. Opferkuch, *Quantitative contributions of IgG, IgM and C3 to erythrophagocytosis and rosette formation by peritoneal macrophages and anti-opsonin activity of dextran sulfate 500,* Eur. J. Immun. *5,* 378 (1975).

[5] I. Gigli and R.A. Nelson, *Complement dependent immune phagocytosis. I. Requirements for C'1, C'4, C'2 and C'3,* Exp. Cell Res. *51,* 45 (1968).

[6] S. Ruddy and K.F. Austen, *C3 inactivator of man: I. Hemolytic measurements by the inactivation of cell-bound C3,* J. Immun. *102,* 533 (1969).

[7] N. Tamura and R.A. Nelson, *Three naturally-occurring inhibitors of components of complement in guinea-pig and rabbit serum,* J. Immun. *99,* 582 (1967).

[8] G.D. Ross, M.J. Polley, E.M. Rabellino and H.M. Grey, *Two different complement receptors on human lymphocytes. One specific for C3b and one specific for C3b inactivator-cleaved C3b,* J. exp. Med. *138,* 798 (1973).

[9] M.P. Dierich, M.A. Pellegrino, S. Ferrone and R.A. Reisfeld, *Evaluation of C3 receptors on lymphoid cells with different complement sources,* J. Immun. *112,* 1766 (1974).

[10] B. Wellek, H. Hahn and W. Opferkuch, *Evidence for macrophage C3d-receptor active in phagocytosis,* J. Immun. *114,* 1643 (1975).

[11] T. Borsos and E.J. Leonard, *Detection of bound C3 by a new immunochemical method,* J. Immun. *107,* 766 (1971).

[12] W. Boyle, *An extension of the ^{51}Cr-release assay for the estimation of mouse cytotoxins,* Transplantation *6,* 761 (1968).