REVIEW

CR3 (CD11b, CD18): a phagocyte and NK cell membrane receptor with multiple ligand specificities and functions

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SUMMARY

The C3 receptor CR3 is expressed on phagocytic cells, minor subsets of B and T cells, and natural killer (NK) cells. It has important functions both as an adhesion molecule and a membrane receptor mediating recognition of diverse ligands such as intercellular adhesion molecule-1 (ICAM-1) and fixed iC3b. The receptor is capable of undergoing an activation event that regulates both its specificity for various ligands and its ability to mediate phagocytosis or extracellular cytotoxicity. Certain bacteria express carbohydrates or lipopolysaccharides (LPS) that can bind to and activate CR3, allowing the receptor to assume its activated state. Soluble β-glucan derived from the yeast *Saccharomyces cerevisiae* is a particularly potent stimulator of CR3, and produces an activated state of the receptor that permits neutrophil phagocytosis of iC3b-coated erythrocytes or $NK_{\star}$ cell cytotoxicity of iC3b-coated tumour cells, that are normally resistant to NK cells.

Keywords CR3 natural killer cell neutrophil C3 receptor monocyte/macrophage phagocytosis tumoricidal activity

Introduction

CR3 is a member of the leucocyte β_2-integrin family that shares the same CD18 β-chain linked to one of three α-chain types (Table 1). The other β_2-integrins are LFA-1 (CD11a, CD18) and CR4 (CD11c, CD18). All three molecules function as intercellular adhesion molecules and membrane receptors with transmembrane signalling ability. CR3 and LFA-1 also share the property of changing ligand-binding specificity and affinity with cellular activation. LFA-1 is expressed on all leucocytes, whereas CR3 is restricted to phagocytic cells, natural killer (NK) cells, and minor subsets of CD5+ B cells and CD8+ T cells. Most cells that express CR3 also express smaller amounts of CR4. Only macrophages and activated B cells express more CR4 than CR3.

This review is focused on CR3 and recent findings showing that CR3 mediates both cellular adhesion and cytotoxic reactions. A key to understanding the functions and multiple ligands of CR3 has been the recognition that CR3 can assume an active state in which new ligand binding sites are exposed and cytotoxic events can readily be triggered (Table 2). In its resting state, CR3 is incapable of mediating cytotoxic reactions, and only some of its adhesion-promoting functions can be demonstrated. Both zymosan (cell wall extract from the yeast *Saccharomyces cereviseae*) and *Escherichia coli* bind to resting CR3 and promote the phagocytically active state of CR3.

Activation of CR3 for cytotoxic reactions

Fixed iC3b on sheep erythrocytes (EC3bi) binds avidly to

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neutrophil, monocyte, and NK cell CR3, but does not trigger any cytotoxic reaction [1–5]. Nevertheless, the phorbol myristate acetate (PMA)-activated CR3 of monocytes [6] or neutrophils [7–9] will mediate phagocytosis of EC3bi, as well EC3dg that are neither bound nor ingested by resting CR3 [10]. Thus, CR3 is capable of mediating phagocytosis following protein kinase C (PKC)-dependent activation. Likewise, neutrophil homotypic aggregation requires PKC for exposure of a CR3 binding site for a counter-receptor on other neutrophils [11,12] recently shown to be L-selectin [13]. Blockade of both PMA-induced phagocytosis [14] and homotypic aggregation [12] by the PKC inhibitor staurosporine suggests a similar activation pathway for these two CR3-dependent functions.

Phagocytosis, degranulation and a respiratory burst stimulated by zymosan and bacteria

Despite the inability of EC3bi to stimulate CR3-dependent functions, fixed iC3b on zymosan and many strains of bacteria stimulates vigorous and CR3-dependent phagocytosis, degranulation, and a respiratory burst [15–18]. CR3 was shown to be capable of direct attachment to unopsonized zymosan, and this attachment to zymosan resulted in phagocytosis and a superoxide burst [19]. When the major carbohydrate components of zymosan were analysed for reactions with monocytes, only the β-glucan and not the mannan was found to produce a response [20]. Comparison of highly purified particles of zymosan-derived β-glucan to the parent zymosan and iC3b-opsonized zymosan indicated that the β-glucan component of zymosan, and not fixed iC3b, was responsible for CR3-dependent re-

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Table 1. The CD18 family of leucocyte β2-integrins

	Receptor specificity	Structure	Available MoAbs	Cellular distribution
LFA-1 (CD11a)	ICAM-1	175-kD α-chain 95-kD β-chain	TS1/22.1.1.13, G-25.2, MHM24, MEM-25, -30, -83, -94, -95	Lymphocytes, monocyte/macrophage, PMN, NK cells
CR3 (CD11b, Mac-1, Mo1)	iC3b, C3dg, LPS, ICAM-1, β-glucan, L-selectin, fibrinogen	165-kD α-chain 95-kD β-chain	Anti-Mac-1, -Mo1, -Leu-15, OKM1, OKM9, OKM10, MN-41, 44, 903, 904, 60.1, Vim-12, 2LPM19c, MEM-169	CD5+ B cells, CD8+ T cells, monocyte/macrophage, PMN, NK cells
CR4 (CD11c, p150,95)	iC3b, fibrinogen	150-kD α-chain 95-kD β-chain	Anti-Leu-M5, L-29, Ki-M1, KB23, Bu-15, K890	Activated B cells, monocyte/macrophage, PMN, NK cells

ICAM-1, Intercellular adhesion molecule-1; LPS, lipopolysaccharide; PMN, polymorphonuclear neutrophil; NK, natural killer.

Table 2. Ligand-binding sites and functions of phagocyte and natural killer (NK) cell CR3 (CD11b, CD18)

	Ligand	Binding site	Site-selective MoAbs	Function
Resting CR3	EC3bi	iC3b	Anti-Leu-15, 903	Adherence
	Zymosan	β-glucan	OKM1	Phagocytosis/respiratory burst
	Escherichia coli	LPS	904	Phagocytosis
Activated CR3	Endothelial cell	ICAM-1	?	Adherence
	Fibrinogen	?	?	Adherence
	Neutrophil	L-selectin	?	Homotypic aggregation
	EC3dg	C3dg	?	Phagocytosis
	K562 cell	?	?	Cytotoxicity

LPS, Lipopolysaccharide; ICAM-1, intercellular adhesion molecule-1.

sponses to iC3b-opsonized zymosan [16]. β-glucan apparently binds to CR3 in a different manner than does fixed iC3b. CR3-dependent responses to β-glucan were blocked by the CR3 α-chain-specific MoAb OKM1, which did not block the attachment of fixed iC3b, whereas another CR3 α-chain-specific MoAb, anti-Leu-15, blocked fixed iC3b attachment, but had no effect on β-glucan responses [19,21]. These data implied a β-glucan binding site in CR3 that was near the OKM1 epitope and capable of triggering CR3-dependent responses, and a second iC3b binding site that was near the anti-Leu-15 epitope and incapable of triggering activation. A similar mechanism has been suggested by the finding that the phagocytosis of certain strains of iC3b-opsonized bacteria was also blocked by MoAbs to CR3 [15,22]. On the other hand, lipopolysaccharide (LPS) on E. coli appears to bind to CR3 at a distinct site from β-glucan and also stimulates CR3-dependent phagocytosis [23]. Moreover, certain fimbriated strains of E. coli stimulate CR3-dependent phagocytosis by way of a mannose-specific lectin on the bacteria that binds to a carbohydrate on CR3 [24].

Activation of CR3 involves a PKC and tyrosine kinase-dependent phosphorylation event

The finding that both neutrophil aggregation and phagocytosis

of EC3bi induced by PMA were blocked by staurosporine suggested that a PKC-associated phosphorylation event was required for CR3 activation [12,14]. Phagocytosis of particulate β-glucan was shown to be associated with a low level of phosphorylation of the β-chain of CR3, and staurosporine was shown to inhibit both phosphorylation and phagocytosis [14]. Although soluble glucans from barley or laminarin bound weakly to CR3 and competed with zymosan [20], they did not stimulate cellular activation. Goldman [25], however, succeeded in isolating a soluble glucan from zymosan using formic acid followed by ethanol precipitation. This zymosan-derived glucan was active in blocking zymosan responses at microgram concentrations. Furthermore, we have recently shown that such a soluble zymosan-derived glucan preparation activates neutrophil CR3, permitting the avid phagocytosis of EC3bi. Not only is glucan-induced phagocytosis of EC3bi prevented by staurosporine, but it is also blocked by genistein or herbimycin A, two tyrosine kinase inhibitors. β-glucan appears to activate CR3 by binding to a lectin-like site in CR3. Soluble glucan labelled with FITC bound to all leucocyte types expressing CR3 and was readily detectable by flow cytometry. Moreover, the staining of monocytes, neutrophils, and NK cells by glucan-FITC was blocked by anti-CR3 [26].

Activation of NK cell CR3 by soluble β-glucan

Although NK cells express CR3, all previously reported investigations of NK cell CR3 had failed to demonstrate any role for CR3 in mediating cytotoxicity. Not only did anti-CR3 fail to inhibit NK cell killing of K562 cells [27], but also NK cell attachment to target cells coated with fixed iC3b did not stimulate cytotoxicity [4,28]. Recognizing the potential of β-glucan to activate neutrophil CR3, Di Renzo *et al.* [29] showed that soluble β-glucan would greatly enhance NK cell-mediated cytotoxicity of K562 cells, and that this enhanced cytotoxicity was blocked by the anti-CR3 MoAb OKM1. In addition, NK cells were shown to be similarly activated by F(ab')₂ fragments of the anti-CR3 MoAb M522 [29]. M522 is the only MoAb to CR3 that has been reported to stimulate a neutrophil respiratory burst [30]. Taken together, these results suggest that M522 binds at or near to the same site in CR3 as does β-glucan. Our own recent experiments have confirmed the finding of Di Renzo *et al.* [29] and in addition have shown that the β-glucan-enhanced killing of K562 cells is blocked by staurosporine, genistein, or herbimycin A. These kinase inhibitors blocked only the enhancement of cytotoxicity stimulated by β-glucan, and not the basal levels of CR3-independent cytotoxicity, and thus the inhibitors did not block NK cell killing reactions mediated by other receptor systems. Of potentially greater importance was the finding that β-glucan stimulated NK cells to lyse iC3b-coated target cells that were normally resistant to NK cells. NK cells incubated with soluble β-glucan avidly lysed sheep or chicken EC3bi, as well as iC3b-opsonized Yac-1 murine tumour cells. Anti-CR3 blocked lysis if added either before the glucan to prevent CR3 activation or after the glucan to prevent activated CR3 attachment to the iC3b-opsonized targets. Target cells lacking iC3b were not killed, and iC3b-coated targets were not killed without soluble glucan. β-glucan also activated CR3 in the same way as PMA, and exposed the binding site for fixed C3dg, thus permitting glucan-activated NK cells to lyse EC3dg. Finally, all lysis was CR3-dependent because it was inhibited completely by staurosporine, genistein, or herbimycin A. Others have previously reported that β-glucan is a potent stimulator of macrophage tumoricidal activity [31]. Various β-glucan preparations have been used for over 20 years in the treatment of certain forms of human cancer [32]. The current data suggest that β-glucan promotes tumoricidal activity of phagocytes and NK cells via activation of CR3 to recognize either fixed iC3b/C3dg or some other unknown ligand such as that expressed by K562 cells.

Conclusions

CR3 is an important phagocyte and NK cell receptor for mediating cellular cytotoxic reactions against target cells bearing specific carbohydrate structures capable of binding to CR3. Fixed iC3b on microorganisms or tumour cells promotes avid attachment of phagocytes or NK cells to the targets via CR3, but is incapable of triggering cytotoxic reactions. However, fixed iC3b on microorganisms bearing the appropriate sugars promotes the avid attachment of CR3-bearing cells to the sugar-containing surface, thereby allowing sugar activation of CR3 followed by cytotoxic reactions. β-glucan derived from zymosan binds to CR3 and activates the receptor via a phosphorylation event involving both PKC and a tyrosine kinase. Such activated CR3 is able to mediate cytotoxic reactions against target cells bearing fixed iC3b.

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