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Pathogen recognition or homeostasis? APC receptor functions in innate immunity

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Abstract

Myeloid cells (macrophages, neutrophils, dendritic cells) express a repertoire of plasma membrane receptors able to recognize all classes of macromolecules. The concept of pattern recognition has emphasized microbial ligands and host defence. However, these receptors play a broader role in tissue homeostasis within multicellular hosts, clearing the extracellular environment of potential undesirable ligands arising endogenously as well as from without. This article will evaluate one of the paradigms that underlie innate immunity. ***To cite this article: S. Gordon, C. R. Biologies 327 (2004).***
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Abbreviations

APC	Antigen presenting cells
IGSF	Immunoglobulin superfamily
MARCO	Macrophage receptor collagenous domain
MØ	Macrophages
NOD	Nucleotide oligomerisation domain
PAMP	Pathogen associated molecular pattern
SR-A	Scavenger receptor, class A
TREM	Triggering receptor expressed in myeloid cells

1. Introduction

From the earliest days of immunological science, the development of complex immune mechanisms has been closely linked to host defence against infection. As part of this, the specificity and memory of adaptive immunity have ruled the day. Earlier emphasis on instruction by antigens, to account for antibody specificity, was replaced by clonal selection of preformed cellular recognition structures, later shown to be due to somatic recombination and diversification of genes in B as well as T lymphocytes. More recently, it has become recognized that innate resistance to infection is germ-line encoded and ancient in evolution. Emphasis has shifted, in part, to the role of receptors on myeloid antigen presenting cells (APC), which then regulate the activation of antigen-specific lymphoid cells. At

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the same time, the problem of discrimination between 'foreign' and 'self' has remained a central concern in host-pathogen interactions and auto-immunity.

The study of APC recognition and responses received impetus from both theory and experimentation. The concept of pattern recognition, economic recognition of conserved molecular microbial structures by a limited number of APC receptors and the discovery of Toll-like receptors (TLR) in *Drosophila* and their role in response to microbial challenge, as well as development, established a new paradigm in innate immunity [1]. Cellular studies on discrimination of particle uptake by phagocytes, and divergent signalling responses to clearance of microorganisms and naturally dying apoptotic cells integrated a body of apparently diverse phenomena [2]. After inevitable initial over-simplification, it is now apparent that the paradigm of innate defence against infection should be reconsidered, in the wider context of tissue homeostasis, as foreseen by Metchnikoff [3].

In this review, I shall consider the role of dual recognition properties by APC plasma membrane receptors for endogenous as well as exogenous ligands, and their relevance to repertoire diversity, selection and immune functions. These cells also contain cytosolic sensors of peptidoglycan breakdown (NOD1,2) that will not be considered here [4].

2. The size and diversity of the APC receptor repertoire

Table 1 shows a selection of plasma membrane receptors implicated in recognition and uptake of various conserved molecular ligands. Somewhat arbitrarily, I exclude specific receptor-ligand interactions which contribute to cell growth, differentiation, adhesion, migration and cellular interactions, although these may well modulate the functions of the endocytic/phagocytic receptors under discussion.

As shown, the list includes receptors for all classes of macromolecules, that is, a rather broader repertoire than found on B and T lymphocytes. Whilst the expression on different myeloid cells varies depending on the particular receptor, many are shared, although regulated independently. Further extensive diversification is achieved by alternative splicing and by post-translational modification, for example, by glycosylation. These receptors are for the most part transmembrane or lipid-anchored glycoproteins, with type-I or -II orientation. A common feature is that these receptors tend to be promiscuous, able to accommodate multiple ligands, often through relatively weak binding, consistent with a role in spatial "pattern recognition". In some cases, complex ligands such as LPS and mannosyl glycoconjugates can bind to several distinct receptors.

A single receptor such as the MR can also bind different ligands through the distinct C-type lectin and cysteine-rich domains [5]; in the case of dectin-1 the single C-type lectin-like domain apparently can bind β-glucans on zymosan as well as uncharacterized ligands on selected T lymphocytes by a glucan-independent binding site [6]. It should be noted that there is little detailed structural information on the ligands or the binding sites of most of these receptors.

3. Multifunctionality and interactions of APC receptors

While the functional emphasis for each of the above receptors may differ, they share common features: binding, endocytosis and/or phagocytosis of modified host constituents or microorganisms, together with intracellular signalling and altered gene expression by the APC. Since many of these receptors are able to respond to both 'foreign' and 'self' or "modified self" ligands, the differences in cellular responses, e.g. in inducing pro- or anti-inflammatory responses cannot be a simple property of the receptor alone, but must depend on involvement of other molecules, either other proteins/receptors in the plasma membrane, or by recruitment of signalling pathways via adaptor molecules. Altered gene expression will therefore vary, depending on the cell itself and additional signals from the microenvironment. Thus a mature MØ can respond very differently to receptor engagement compared with a primed neutrophil or an immature DC, which all display efficient phagocytic ability.

These receptors do not function in isolation. A particle such as a bacterium or apoptotic cell will express a multiplicity of different ligands that engage a range of different receptors. Other modulatory molecules regulate exposure on the surface of the particle, as well as distribution and availability of recep-

Table 1
Selected pattern recognition receptors of APC

Receptor	Characteristics	Expression	Selected Ligands	Comments	Selected References
TLR	Leucine-rich repeat extracellular, TIR domain intracellular	Myeloid & others (inducible)	Lipopolysaccharide Lipoproteins/flagellin synthetic CpG, ds RNA, ?hsp	Diverse receptors Signalling sensors Pro-inflammatory & other effects	[12]
CD14	GPI-anchored	Myeloid	LPS-binding protein, lipoproteins & apoptotic cells	LBP can also bind G^+ peptidoglycan	[13,14]
CR3	β2 integrin heterodimer	Myeloid	ICAM-1, factor X, LPS, zymosan, apoptotic cells	Opsonic & non-opsonic phagocytosis Inflammatory recruitment	[15]
SR-AI/II	Collagenous, helical with or without Cysteine-rich domain (Type II)*	Selected MØ, DC & endothelium	Selected polyanions Modified host lipoproteins Apoptotic cells, Calreticulin, gp96 hsp chaperone *E. coli* (lipid A) *S. aureus* (lipoteichoic acid), *Neisseria* sp	Adhesion endocytosis & phagocytosis	[16,17]
MARCO	Collagenous, helical with Cysteine-rich domain (Type II)	Selected MØ esp. marginal zone MØ	*E. coli, S. aureus* Selected B lymphocytes	Inducible via TLR	[18]
MMR	C-type multilectin Cysteine-rich domain	Selected MØ, DC & endothelium	Man, fuc, GlcNac Glycoconjugates Lysosomal hydrolases ?*Klebsiella, Candida albicans*, HIV-1, Some LPS	Bi-functional lectin Ag clearance to spleen & lymph node	[5,19]
DC-SIGN	C-type lectin (Type II)	DC	ICAM-3 HIV-1, Dengue virus, mTb	HIV-1 delivery to CD4 T cells	[20]
Dectin-1	C-type lectin-like ITAM in cytoplasmic tail (Type II)	Myeloid	β-glucan Selected T cells (sugar independent)	Zymosan & fungal phagocytosis Collaboration with TLR Co-stimulator for T cell activation	[6,8]
TREM 1,2	Single IgSF	Myeloid cells	?Microbial, anionic (2-astrocytoma)	DAP-12 signal	[21,22]
Sialoadhesin (Siglec 1)	Multi IgSF	Selected MØ esp. marginal metallophilic MØ	Sialic acid-glycoconjugates, host, *Neisseria*	Cell–cell interactions	[23]
CD91	α/β subunits with associated protein	MØ & fibroblasts, hepatocytes, keratinocytes	α_2 macroglobulin/proteinase complexes Calreticulin	Clearance	[17]
CD163	Multiple SRCR domains	MØ	Haptoglobin–haemoglobin complex	Glucocorticoid inducible Clearance	[24]
CD1	MHC-IB	DC	Gangliosides, e.g., Mycobacteria ?endogenous	APC to NKT cells	[25]

* Transmembrane glycoproteins with type-II orientation are indicated, otherwise type I.
TREM – Triggering receptor expressed on myeloid cells.

tors. Experimental models have begun to define interactions among different receptor molecules. For example, CD14 and TLR4, as well as other proteins such as MD2 and MyD88, form a protein-lipid complex necessary for capture and signal generation [7]. In the case of zymosan uptake, initial binding via Dectin-1, the main β-glucan receptor, is linked to TLR 2/6 function, required to induce TNFα secretion [8]. The cytoplasmic tail of surface-expressed Dectin-1 is necessary and sufficient for TLR coupling. In other cases, such as SR-A I/II function, the phagocytosis of unopsonised *Neisseria meningitidis* (NM) is independent of lipid A, a reported ligand for TLR stimulation and TNFα release [9]. The induction of MARCO by NM, however, does depend on TLR involvement (S. Mukhopadhyay, L. Peiser and S. Gordon, J. Leuk. Biol., In press).

A further level of complexity remains to be defined. Other receptor-ligand interactions than those already noted also influence phagocytic receptor function (CD47-SHPS, SIRPα) or cell activation (CD200/CD200R) [10]. Finally, the deposition of opsonins (antibody, complement, fibronectin, thrombospondin, milk fat globulin EGF-factor 8 [MFG-E8]) [11] will enhance uptake of particles, and it should be envisaged that potent anti-opsonins that remain to be defined could limit uptake.

Taken together, it can be seen that the discrimination between friend (albeit potentially noxious, modified or abnormal) and foe, and resultant responses (clearance, inflammation, T cell activation, antibody production) is not straightforward. "Safe" clearance of apoptotic cells is accompanied by anti-inflammatory responses by MØ, whereas cross priming and activation of T lymphocytes may follow uptake by immature DC.

4. How did the APC repertoire evolve?

The appreciation of the dual nature of phagocytic and endocytic ligand recognition may help to clarify the process of evolution of the innate immune system. By placing the emphasis on endogenous as well as on exogenous microbial ligands (the PAMPs of Janeway and Medzhitov) [1], it is possible to suggest a Darwinian model of receptor diversification by intrinsic genetic (germ line) mechanisms and selection by homeostatic host requirements as well as by fitness in host defence against pathogens. Conventional gene duplications and mutations may be sufficient, although it is possible to incorporate genetic elements from retroviruses and even from intracellular bacteria. No one would dispute that many viruses have co-opted normal physiologic surface molecules of host cells for attachment and entry, similarly, bacteria can take advantage of receptors such as CR3 and SR-A for safe entry. Even the uptake of extracellular bacteria by macrophages can provide a haven from extracellular killing mechanisms such as lysis by complement.

Claude Bernard proposed that the multicellular host depends on a stable, protected "milieu intérieur". Resident myeloid cells and their receptors provide a responsive dispersed (blood and tissue) system to clear physiological molecules (e.g. lysosomal hydrolases), protein aggregates (e.g. protease–inhibitor and haptoglobin–haemoglobin complexes), denatured molecules (e.g. modified/oxidized lipoproteins) and senescent/apoptotic cells from the extracellular space, and remove them silently by degradation. Clearance of calreticulin and other chaperones performs a similar role for stress-induced or misfolded molecules released extracellularly. The TLR can be seen to have been co-opted for inflammatory signalling from an original developmental function (most *Drosophila* TLR do not have any host defence function). It is thus perhaps not surprising that when lymphoid cells acquired a somatic recombinatorial capacity they would still depend on processing of potential antigens by the APC. In parallel, the diversity of MHC molecules evolved in order to present peptides to the more specialized lymphocytes. Early examples of limited requirements for MHC/MHC-like diversity are found on NK, NKT and $\gamma\delta$ T cells. Monocytes/MØ also express a very wide range of NK-like inhibitory and activatory molecules, including many orphan C-type lectin-like and immunoglobulin superfamily receptors (IGSF), but the role of MHC restriction in their intercellular interactions remains unknown.

5. Conclusion

I believe that a shift of emphasis from exogenous to endogenous ligands and from pathogen interactions to homeostatic requirements, will restore the role of the innate immune system as primarily a self-regulating,

physiologic system, with defence as a secondary, albeit vital, by-product. In a sense it is the immunological counterpart of the perennial debate between innate endowment and environmental pressures of selection. Progress in this field will depend on definition of individual receptor structures, their interactions and identification of naturally occurring ligands within and outside the host.

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