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Review

A parasitic helminth-derived peptide that targets the macrophage lysosome is a novel therapeutic option for autoimmune disease

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ABSTRACT

Parasitic worms (helminths) reside in their mammalian hosts for many years. This is attributable, in part, to their ability to skew the host's immune system away from pro-inflammatory responses and towards anti-inflammatory or regulatory responses. This immune modulatory ability ensures helminth longevity within the host, while simultaneously minimises tissue destruction for the host. The molecules that the parasite releases clearly exert potent immune-modulatory actions, which could be exploited clinically, for example in the prophylactic and therapeutic treatment of pro-inflammatory and autoimmune diseases. We have identified a novel family of immune-modulatory proteins, termed helminth defence molecules (HDMs), which are secreted by several medically important helminth parasites. These HDMs share biochemical and structural characteristics with mammalian cathelicidin-like host defence peptides (HDPs), which are significant components of the innate immune system. Like their mammalian counterparts, parasite HDMs block the activation of macrophages *via* toll like receptor (TLR) 4 signalling, however HDMs are significantly less cytotoxic than HDPs. HDMs can traverse the cell membrane of macrophages and enter the endolysosomal system where they reduce the acidification of lysosomal compartments by inhibiting vacuolar (v)-ATPase activity. In doing this, HDMs can modulate critical cellular functions, such as cytokine secretion and antigen processing/presentation. Here, we review the role of macrophages, specifically their lysosomal mediated activities, in the initiation and perpetuation of pro-inflammatory immune responses. We also discuss the potential of helminth defence molecules (HDMs) as therapeutics to counteract the pro-inflammatory responses underlying autoimmune disease. Given the current lack of effective, non-cytotoxic treatment options to limit the progression of autoimmune pathologies, HDMs open novel treatment avenues.

Contents

Abbreviations: HDMs, helminth defence molecules; HDPs, cathelicidin-like host defence peptides; TLR, toll like receptor; v-ATPase, vacuolar H^+-ATPase; DCs, dendritic cells; Th2, T helper 2; TNF, tumour necrosis factor; IFN, interferon; MS, multiple sclerosis; RA, rheumatoid arthritis; T1D, type 1 diabetes; NOD, nonobese diabetic; SLE, systemic lupus erythematosus; EAE, experimental allergic encephalomyelitis; FhHDM-1, *Fasciola hepatica* helminth defence molecule; CRAMP, cathelicidin-related antimicrobial peptide.

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Introduction

The endosomal/lysosomal pathway plays a central role in determining the functions of innate immune cells, such as macrophages. Endosomes/lysosomes can limit the localisation of internalised material and signalling motifs, and can also determine the processing of both to modulate the phenotype/function of innate immune cells (Bright et al., 2005; Luzio et al., 2007; Watts, 2012). Specifically, these innate immune cells endocytose/phagocytose pathogens and then process (to varying degrees) antigens, *via* the endosome/lysosome system (Bright et al., 2005; Luzio et al., 2007). Accordingly, innate immune cells are reliant upon the endosome/lysosome components to determine the responses that define the resultant adaptive immune response. Aside from extracellular recognition, nucleic acids and pathogens (such as viruses and bacteria) are also recognised within endosome/lysosome organelles and this induces intracellular signals, which modulate innate immune cell function (Blasius and Beutler, 2010). These phenomena suggest that regulation of the environment within endosomes/lysosomes will modulate immune responses. Many factors (such as pH, lipid composition and membrane potential) are involved in fine-tuning the environment within the endosome/lysosome compartments to elicit signalling pathways. Alterations to any of these factors greatly affect immune cell function, and thus the ultimate immune response generated. As a consequence, these endosomal/lysosomal regulatory factors are being targeted for the development of anti-inflammatory drugs to treat pro-inflammatory and autoimmune disease (Kobayashi et al., 2013; Ge et al., 2014). This review focuses on the role of the lysosomal mediated activities of macrophages in the development of the pro-inflammatory immune responses that cause autoimmune disease, and the potential of helminth defence molecules (HDMs) to prevent these pro-inflammatory responses. Given the current lack of effective, non-cytotoxic treatment options to limit the progression of autoimmune pathologies, HDMs open novel treatment avenues.

The lysosome is central to the biological function of the macrophage

Once thought of as only intracellular organelles for the degradation of endocytosed material, lysosomes are now regarded as multi-functional organelles involved in many cellular processes, such as secretion, plasma membrane repair, nutrient sensing, and cell signalling. Many of these activities are dependent upon the acidic pH within the lumen (Settembre et al., 2013), which is primarily controlled by the action of vacuolar H^+-ATPase (v-ATPase), a transmembrane, multimeric protein complex that pumps protons into the lysosomal lumen, using energy generated from the hydrolysis of ATP (Beyenbach and Wieczorek, 2006; Mindell, 2012). This characteristic acidic environment is optimal for the activity of the sixty lysosomal hydrolases (including proteases, lipases, glycosidases, and nucleases), which mediate many of the biological functions of the macrophage (Bainton, 1981). Of these hydrolases, the cathepsin family of cysteine proteases are the best characterised to date, and the cathepsins B, C, H, and L are among the most abundant lysosomal hydrolase enzymes (Rossi et al., 2004).

Degradation of endocytosed macromolecules

Macrophages play a central role in the activation of helper ($CD4^+$) T cells, *via* the uptake, processing and presentation of antigenic peptides on MHC class II molecules, along with the secretion of cytokines. Macrophages internalise extracellular components by endocytic or phagocytic mechanisms, which culminate in the formation of endolysosomal hybrids (resulting from fusions between late endosomes and lysosomes), which facilitates the exchange of intra-organelle contents. This fusion process renders the internalised molecules susceptible to degradation by the lysosomal hydrolases (Bright et al., 2005; Luzio et al., 2007), which operate most efficiently at acidic pH (Talloczy et al., 2008; Mindell, 2012). In addition, the acidic pH of the lysosomal lumen causes the relaxation of tertiary structures, making proteins more accessible to the hydrolase enzymes (Ohkuma et al., 1982). The resultant antigenic peptides are then loaded onto MHC-II molecules, transported to the cell surface, and presented to $CD4^+$ T cells (Apostolopoulos et al., 2008; Harding and Ramachandra, 2010; Watts, 2012). The levels of both presented peptide and co-stimulatory signals determines the ultimate immune outcome; tolerance *versus* (auto) immunity.

Global inhibition of cysteine protease enzymes, using E-64, completely suppresses antigen processing and presentation *in vivo* (Katunuma et al., 2003). However, each lysosomal hydrolase appears to play a distinct role in antigen processing, and, accordingly, the ultimate immune response generated. For example, specific inhibition of cathepsin D blocks the priming and expansion of antigen specific T cells, whereas inhibition of cathepsin B skews the phenotype of immune responses from a T helper 2 (Th2) to a Th1 type (Zhang et al., 2000).

Regulation of innate immune cell signalling

In addition to inducing T cell responses, the liberation of bacterial-derived ligands by pH-dependent lysosomal proteases is important for the engagement of toll like receptor (TLR) sensing mechanisms. Within the endolysosomal compartment, TLR3, TLR7, TLR8, and TLR9 mediate the induction of pro-inflammatory responses to viral and bacterial nucleic acids (Blasius and Beutler, 2010). In the absence of lysosomal acidification, and the consequential activation of proteases, many of the immune-stimulatory ligands of phagocytosed bacteria would remain inaccessible, and, hence, unable to fully activate TLR-dependent responses (Ip et al., 2010). Furthermore, for TLRs located in the lysosomal

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