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REVIEW

Parasite cysteine proteinase interactions with α2-macroglobulin or kininogens: differential pathways modulating inflammation and innate immunity in infection by pathogenic trypanosomatids

Julio Scharfstein*

Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, C.C.S., Ilha do Fundão, Rio de Janeiro 21949-900, RJ, Brazil

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Abstract

Plasma extravasation is a common endothelium response to tissue injury provoked by pathogens. Herein I will review studies showing that host proteinase inhibitors (e.g., α2-macroglobulin (α2M) or kininogens) interact with protozoan cysteine proteinases (CPs) in extravascular infection sites, linking inflammation to innate immunity by different mechanisms. Using human monocytes as antigen presenting cells, we first demonstrated that α2M entrapment of cruzipain, a *Trypanosoma cruzi* CP, reduced the activation threshold of cruzipain-specific CD4 T cells due to facilitated uptake of α2M–cruzipain complexes by the multiscavenger receptor (CD91). More recently, studies of the mechanisms underlying inflammation elicited by *T. cruzi* revealed that kininogens, once bound to glycosaminoglycans, are not able to efficiently inactivate cruzipain via their inhibitory cystatin-like domains. Instead, we found that cruzipain readily processes surface-bound kininogens, liberating bioactive kinins. Acting as paracrine hormones, kinins vigorously activate host cells through bradykinin (BK) receptors, thus stimulating endocytic uptake of the pathogen. Rather than unilaterally enhancing parasite infectivity, the liberated kinins activate innate immunity by potently stimulating dendritic cell maturation via the BK B_2 receptor. The discovery of chagasin, a novel family of endogenous inhibitors expressed by trypanosomatids, is likely another regulatory player involved in the dynamics of the inflammatory response.

Keywords: Leishmaniasis; *Trypanosoma cruzi*; Innate immunity; Kinins; α2-macroglobulin; CD91; Cysteine proteases

Abbreviations: α2M, α2-macroglobulin; ACE/kininase II, angiotensin converting enzyme; APC, antigen presenting cells; BK, bradykinin; B_1R, kinin B_1 receptor; B_2R, kinin B_2 receptor; CD91/α2MR, α2-macroglobulin receptor; CP, cysteine proteinases; cruzipain, major cysteine proteinase of *T. cruzi*; DCs, dendritic cells; DEC-205, scavenger DC205; ECM, extracellular matrix; GPCRs, G protein-coupled receptors; G_s, stimulatory G protein; G_i, inhibitory G protein; HK/LK, high and low molecular weight kininogen; kininase I, carboxypeptidase M/N; NEP, neutral endopeptidase; NKT, natural killer T cells; PAF, platelet activating factor; PLC-β, phospholipase C-β; PRR, pattern-recognition receptors; TLR, Toll-like receptors

*Tel.: +55 21 2209 6591; fax: +55 21 2280 8193.
E-mail address: scharf@biof.ufrj.br (J. Scharfstein).

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Introduction: multifunctional scavenger receptor (CD91) promotes antigen presenting cell (APC) uptake of parasite proteinases

It is well established that in situations when antigen availability is limited, receptor-mediated uptake by APCs optimizes processing and peptide presentation to T cells. In this review, I will initially focus on the functional role of CD91, originally designated as LRP, the lipoprotein receptor related protein, and also known as the multiscavenger α2-macroglobulin receptor (α2MR) (Herz and Strickland, 2001). Of possible relevance for maintenance of tolerance states in the steady state, CD91 promotes macropinocytosis and uptake of apoptotic bodies by APC through ligand interactions with C1q and the mannose binding lectin (Ogden et al., 2001). In the rheumatoid joint, however, CD91 mediates internalization of autologous antigens chaperoned by inducible heat shock proteins (Martin et al., 2003), thus contributing to pathological cross-priming. CD91 promotes the clearance of α2-macroglobulin (α2M) molecules bound to a variety of proteinases and cytokines. Conformational changes induced by entrapped proteinases lead to the exposure of CD91 recognition sites in the bound α2M (Adlakha et al., 2001). α2M–proteinase complexes are then rapidly internalized by the APCs via CD91-dependent endocytosis. Interest in the innate role of CD91 in humans was rejuvenated by the recent identification of a small subset of dendritic cells (DCs) in the bloodstream (Hart et al., 2004).

Analysis of antigen uptake by monocytes from Chagas' disease patients offered a first precedent that parasite cysteine proteinase (CP) may be internalized and processed by APCs via the α2M/CD91 pathway (Morrot et al., 1997). We have addressed this issue with cruzipain, the major CPs of *Trypanosoma cruzi* (Cazzulo et al., 1989; Eakin et al., 1990; Murta et al., 1990). Essentially required for parasite infectivity and intracellular growth in mammalian cells (Aparicio et al., 2004; Engel et al., 1998; Meirelles et al., 1992; Scharfstein et al., 2000; Scharfstein, 2003), cruzipain was recently characterized as a therapeutic target in Chagas' disease (Engel et al., 1998; McGrath et al., 1995; McKerrow, 2005). Prior to its characterization as a lysosomal CP (Murta et al., 1990), serological screens performed with cohorts of Chagas' disease patients have identified cruzipain as a dominant *T. cruzi* antigen (Duschak et al., 2001; Scharfstein et al., 1983, 1985, 1986). Mature cruzipain consists of a single polypeptide chain folded as two independent domains linked to each other by a polythreonine-rich hinge (Aslund et al., 1991). Antibodies from chagasic serum are mostly directed against the long and heavily glycosylated C-terminal extension (Scharfstein et al., 1983, 1985) while the human MHC-class II restricted T cell epitopes or MHC-class I epitopes seem to preferentially map to the catalytic domain (Arnholdt et al., 1993; Fonseca et al., 2005; Svensjö et al., in press).

We became interested in studying the consequences of cruzipain interaction with α2M while inspecting myocardial sections derived from patients with severe forms of Chagas' disease. Immunohistochemistry showed presence of cruzipain antigen deposits amidst cellular infiltrates enriched with $CD91^+$ mononuclear cells (Morrot et al., 1997). Although not formally excluding a secondary role for autoimmunity (Engman and Leon, 2002), other groups showed that intensity of inflammatory infiltrates correlates with presence of parasite antigens in the myocardium (Palomino et al., 2000). Efforts to characterize the T cell subsets infiltrating the heart appointed TNF-α producing CD8 T cells as effectors of immune tissue damage (Reis et al., 1993). More recently, cruzipain-specific and FL-120 antigen-specific CD8 T cell clones restricted by HLA-A*0201 were detected in the peripheral blood of chronic chagasic patients (Fonseca et al., 2005). In the same study, the authors used tetramer staining with a cruzipain 60–68 peptide to identify an intra-cardiac CD8 clone isolated from a chagasic patient. Mice studies revealed that vigorous anti-parasite immune responses effectively prevent parasite outgrowth in multiple tissues (Kumar and Tarleton, 2001), but the parasites are not eradicated for reasons that are not fully understood (Cummings and Tarleton, 2004; Leavey and Tarleton, 2003).

It is still unclear how extracellular parasites may contribute to chronic myocardiopathy. Once released to extracellular spaces, amastigotes tend to accumulate in the proximity of primary infection foci, because they