Siglecs take a TOLL on inflammation: deciphering the Hsp70 riddle

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The manuscript by Fong *et al* (2015) in this issue of *The EMBO Journal* addresses an ongoing question in immunology: are extracellular HSPs endogenous inflammatory signals or are they in fact built-in inhibitors of those same functions? In a highly original series of experiments, they identify novel Hsp70 receptors in monocytes as members of the Siglec family. Even more significantly, two Siglec family members transmitted divergent responses to Hsp70, with Siglec-5 dampening inflammation while Siglec-14 triggering the inflammatory cascade. These findings therefore go some way in addressing the open question: is Hsp70 pro- or anti-inflammatory and leaving us with the answer yes.

See also: JJ Fong *et al* (November 2015)

The primary role of the heat-shock protein (HSP) families is to fold the intracellular proteome. That is what being a molecular chaperone is all about. However, as with many families in this era, these proteins have extra jobs besides their main employment as chaperones. HSPs are released from cells and take part in cell–cell signaling interactions. They thus play roles in coordinating inflammation and immunity, wound healing, tumor metastasis, and other processes (Asea *et al*, 2000; Annamalai *et al*, 2009; Weng *et al*, 2013; Jayaprakash *et al*, 2015). Their immune properties have, however, proven to be bewilderingly ambiguous. While there seems little doubt that HSPs can transport antigenic peptides and mediate their presentation by antigen presenting cells (APC), their allegiance, for or against inflammation, is controversial

(Murshid *et al*, 2010). Some studies ascribe a role to HSPs as danger signals that trigger inflammation, while for others these proteins seem frankly inhibitory to this process (Asea *et al*, 2000; Sijts & van Eden, 2012). This issue is complicated by the findings that intracellular HSP levels become increased in, are processed by, and presented on the cell surface of inflammatory cells, triggering regulatory T-lymphocyte activity (Broere *et al*, 2011). One would predict that the resolution of wound healing and mediation of metastasis might involve, at least partially, their anti-inflammatory properties. However, under contrived conditions such as when HSPs are used in cancer therapy combined with inflammatory cell killing, these proteins become strongly immunogenic and proinflammatory (Kottke *et al*, 2007; Weng *et al*, 2013).

Clues as to the responses of cells to extracellular chaperones may lie in the nature of cell surface HSP receptors. HSP receptors include scavenger receptor family members as well as LDL receptor CD91 (Binder *et al*, 2000; Theriault *et al*, 2006). However, none of these proteins is listed among the inflammatory receptors. They appear to have in common ancestral connections to CED1, the apoptotic cell corpse engulfing receptor discovered in *C. elegans* (Zhou *et al*, 2001). The connection between cell body uptake and antigen uptake through association with HSPs may involve the efficient internalizing properties of these receptors. The recent studies by Fong *et al* (2015) shown in this issue of *The EMBO Journal* bring into play another receptor type, the *sialic acid-binding immunoglobulin-like lectins* (Siglec) (Ali *et al*, 2014; Fong *et al*, 2015). The Siglecs are a large family

that share the ability to bind conjugated sugar (sialic acid) residues in the glycocalyx of cells and suppress local inflammation. This is achieved through an intracellular ITIM (immunoreceptor tyrosine-based inhibitory motif) that is phosphorylated by tyrosine kinases and then recruits proteins that inhibit inflammation (Chen *et al*, 2014). It was recently shown that many of the Siglecs, both human and rodent, suppress inflammation by directly associating with Toll-like receptors (TLR), the primary gateway proteins for the inflammatory response (Chen *et al*, 2014). This field was shown to intersect with the HSP world by the demonstration that two family members found only in primates, Siglec-5 and Siglec-14, could bind directly to Hsp70 (Fong *et al*, 2015; Fig 1). More intriguingly, although each of these proteins contained similar extracellular Hsp70 binding domains, their intracellular regions enclosed structures with contrasting immunological functions. Siglec-5, in accordance with Siglec family traditions, contains an ITIM domain. However, Siglec-14 was shown to stand alone in incorporating a transmembrane domain that binds adapter proteins such as DAP12 containing immune-activating ITAM (immunoreceptor tyrosine-based activating motif; Fong *et al*, 2015; Fig 1). These two Siglec family members have thus evolved the capacity to bind Hsp70 as well as exhibiting a Ying-Yang type duality in being either for (Siglec-14) or against (Siglec-5) inflammation. Siglec-5 expression in human monocytes reduced inflammatory signaling and cytokine secretion, while Siglec-14 had the opposite properties and boosted signaling and release of IL-8 and TNF- α . The mechanisms behind these contrasting properties have not been

Figure 1. Inflammatory balancing act in response to extracellular Hsp70.

We depict the influence of Siglec receptor family members in mediating inflammatory balance in monocytes by either suppressing TLR signaling through ITIM-containing Siglec-5 or boosting such inflammatory signaling through a complex of Siglec-14 and ITAM-containing DAP12.

described yet, although in accord with earlier studies on other Siglecs, one envisages direct association of these proteins with TLR family members and either a slowing (Siglec-5) or acceleration (Siglec-14) of inflammation (Fong *et al*, 2015).

Does all this make things clearer in the HSP field? Indeed, for understanding how HSPs affect human tissues, the discovery of the *Janus*-like qualities of these Siglecs can neatly explain some of the contradictory properties of extracellular HSPs. However, a dilemma still remains for rodent studies. For instance, both bacterial LPS and Hsp90 or Hsp70 bind to the SREC-I receptor in murine macrophages—but only LPS leads to inflammatory cytokine production via this route (Murshid *et al*, 2015). LPS–SREC-I complexes sequester TLR4 within lipid raft microdomains and mediate inflammatory signaling. It would appear, however, that some activity restrains TLR4 signals emanating from SREC-I in the presence of HSPs (Murshid *et al*, 2015). One potential moderating factor was scavenger receptor A1 (SRA1), an HSP binding protein that can quench TLR4 signaling (Wang *et al*, 2007).

However, blocking SRA1 binding failed to restore TLR4-mediated signaling in cells exposed to Hsp90. Siglec-5 and Siglec-14 have been found only in primates, and a role for them could therefore be ruled out (Fong *et al*, 2015). Another HSP-triggered anti-inflammatory pathway must thus be envisaged for the mouse. Whether this mechanism involves the rodent Siglec family or alternative receptor types remains to be determined.

What will be the next step in this saga? For deployment of HSPs in cancer vaccines, organ transplant, wound healing, and cancer metastasis studies, it is desirable to know which receptors mediate the effects. This would permit us to predict the potential involvement of inflammation in these phenomena and aid in the design of immunotherapy protocols involving HSP vaccines. Thus, the recent emergence of Siglec-5 and Siglec-14 as HSP receptors is highly significant (Fong *et al*, 2015). In addition, we wish to know which tissues express what HSP receptors and whether their levels go up or down in physiological regulation and pathological conditions. The next few years thus offer exciting opportunities to solve the riddle of HSPs in immunity.

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