Autoimmunity heats up

Andrew E Gelman & Laurence A Turka

A new study strengthens the view that heat-shock proteins serve as alarm bells for the immune system. Hsp70 appears to influence the immune response to endogenous stimuli, and may serve as a trigger for autoimmunity (pages 1469–1476).

A healthy immune system differentiates between self and non-self molecules. During an autoimmune response this distinction is lost, resulting in inflammation and tissue destruction. What triggers this breakdown? *In vitro* studies have suggested that heat-shock proteins (HSPs) released by necrotic cells might prompt otherwise quiescent immune cells to become autoreactive. In this issue, Millar *et al.* move these studies *in vivo*, demonstrating that Hsp70 can induce autoimmunity in a mouse model[1]. Their data reveal how therapies might be developed to specifically target autoimmune processes while bypassing other immune responses necessary for host defense.

During development in the thymus, T cells are checked for self-reactivity, and those with potentially autoreactive T-cell receptors are removed from the repertoire. However, the system is not infallible. Some T cells with the potential to react to self antigens inevitably escape into the periphery.

Several additional mechanisms in the periphery keep potentially autoreactive T cells in check. These include restricted expression of costimulatory molecules needed for T-cell activation, and populations of regulatory T cells that suppress T-cell-mediated responses. Nevertheless, in some patients, a combination of genetic and environmental factors leads to the activation of potentially autoreactive T cells, and autoimmune-mediated tissue destruction[2].

Given these considerations, understanding what controls initial T-cell activation may be the key to unraveling autoimmunity. Naive T-cell activation requires antigen to be presented by specialized antigen-presenting cells (APCs), which also provide important signals through secreted cytokines and cell-surface costimulatory molecules (such as members of the B7 and tumor necrosis factor families). Immature APCs efficiently capture antigen, but only upon maturation do they effectively provide the costimulatory signals and cytokines needed to initiate and propagate T-cell responses.

APC maturation can be triggered through Toll-like receptors (TLRs), highly phylogenetically conserved sensors that monitor the local environment for inflammatory 'danger' signals. TLRs were originally thought to recognize only pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS)[3]. But it is now known that TLRs can also respond to endogenous signals released by injury or necrotic cell death[4]. Like PAMPs, these endogenous danger signals may stimulate T-cell responses by enhancing APC maturation[5].

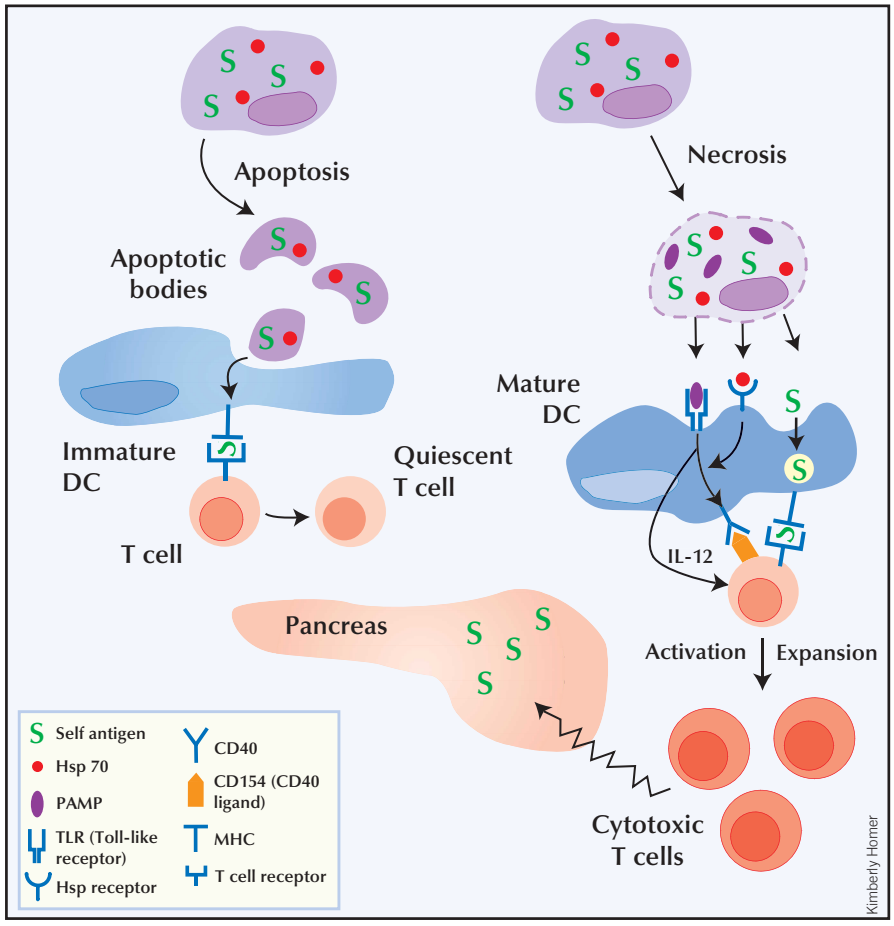

Figure 1 Death and the dendritic cell. Cells die in two distinct ways that have opposing effects on DC maturation. Apoptotic death prevents release of endogenous inflammatory signals, and instead generates apoptotic bodies—small vesicular cell fragments that do not induce DC maturation. In contrast, necrotic death due to infection can lead to the corelease of PAMPs and endogenous signals such as Hsp70. PAMPs then interact with TLRs, leading to the upregulation of costimulatory molecules such as CD40 and proinflammatory cytokines such as IL-12. Millar *et al.* provide evidence that Hsp70 induces maturation of DCs *in vivo*, which can trigger autoimmunity. Hsp70 probably binds multiple receptors, but Hsp70-mediated DC maturation has also been reported to be TLR dependent and to stimulate antigen presentation. Mature DCs then stimulate T cells to divide and differentiate into immune effector cells such as cytotoxic T cells. If the T-cell effectors recognize a particular self antigen, then organs bearing that self antigen can be targeted for tissue destruction.

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Hsp70, a protein upregulated during cellular stress, is a molecular chaperone that facilitates protein folding and antigen presentation within cells[6]. *In vitro* findings show that when released by necrotic cells, Hsp70 has a quite different role. It acts like a danger signal by enhancing maturation of dendritic cells (DCs), the most potent type of APC[7]. These studies show that Hsp70 can be a trigger for immune activation.

Millar *et al.* asked the next logical question: does Hsp70 also ring an alarm for the immune system *in vivo*? To do this, they used engineered mice expressing gp in the pancreas. gp is a viral nuclear protein from lymphocytic choriomeningitis virus. The mice also expressed a T-cell receptor specific for gp33, a region of gp. The authors found that these mice remained healthy even when injected with gp33 peptide. However, when the peptide was coinjected with Hsp70, the mice rapidly developed autoimmune diabetes, associated with inflammation and infiltration of T cells into the pancreatic islets.

Unlike LPS, Hsp70 did not upregulate costimulatory molecules on DCs. Nevertheless, Hsp70 helped APCs become better stimulators for T cells, although exactly how is unknown.

Although LPS coinjected with gp33 was also able to induce diabetes, the authors provide evidence that LPS and Hsp70 act through different routes. Diabetes mediated by gp33 and Hsp70 required the APC costimulatory ligand CD40, whereas diabetes induced by LPS and Hsp70 did not. Specifically, in the absence of CD40, Hsp70 was unable to upregulate production of the inflammatory cytokine IL-12 by DCs. In contrast, LPS-stimulated DCs seemed to release IL-12 in a manner independent of CD40.

The work by Millar *et al.* elegantly connects two autoimmunity paradigms for the first time *in vivo*. First, it is now clear that endogenous signals, which are known products of necrotic cells, promote autoimmune responses. Second, it is the response of APCs to these endogenous signals that triggers autoimmunity.

The study also leaves open some important questions. For example, there is no clear explanation for the finding that Hsp70-stimulated DCs require CD40 for IL-12 synthesis, whereas LPS-stimulated DCs do not. Although one report shows Hsp70 binding to CD40 (ref. 8), other studies show that TLRs mediate APC responses to Hsp70 (ref. 9). Moreover, APC-mediated IL-12 production has been shown to occur through the same toll-like receptor, TLR4, in response to both LPS and Hsp70 (ref. 9). Thus, the story may be more complicated than previously appreciated. The next step, therefore, may be to see whether Hsp70-mediated autoimmune responses occur in the transgenic mouse model in a TLR4-deficient background.

The initial inflammatory conditions that produce the endogenous danger signals leading to autoimmunity are also unclear. Normal cell death occurs by apoptosis, which inhibits DC maturation[10] (**Fig. 1**). The release of endogenous inflammatory signals (like Hsp70) requires necrotic cell death, such as from infection or injury. One notion is that exogenous PAMPs displayed by infectious agents create the initial conditions for autoimmunity, and this in turn leads to necrotic cell death, which perpetuates the autoimmune response. Selected clinical observations fall in line with this notion, as does the finding that the transgenic mice used by Millar *et al.* can also acquire diabetes through infection with lymphocytic choriomeningitis virus[11]. Whether or not endogenously generated danger signals contribute to perpetuation of the inflammatory process in autoimmunity is not known, although the Millar study indicates that this is a real possibility.

Despite these limitations in our knowledge, one of the most exciting findings of the new work is that CD40 is necessary for autoimmunity induced by Hsp70, but not by LPS. This finding suggests that APCs can discriminate between exogenous and endogenous maturation agents via CD40 signaling. If future studies support this observation, CD40 and its signaling pathway could become targets for the therapeutic intervention of autoimmune disease, perhaps enabling selective sparing of host antipathogen responses.

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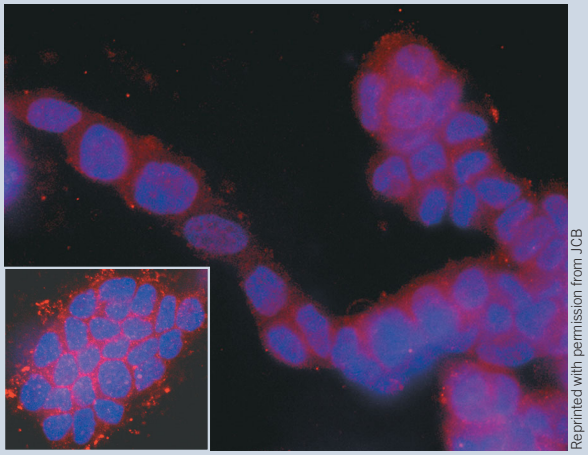
Push me, pull you

In the breast, cells are faced with the existential question common to many cells: differentiate or proliferate? In the 10 November *Journal of Cell Biology*, Michele Wozniak *et al.* consider this question in an *in vitro* system of epithelial breast differentiation. They examine how the flexibility of the extracellular matrix influences this critical decision.



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It has been known for years that breast epithelial cells differentiate into tubules (at right) if the cells are cultured in a three-dimensional collagen matrix floating in medium. But if the matrix is made more rigid by attachment to a surface, or more dense by increasing the collagen concentration, proliferation is favored. How do breast cells sense the flexibility of their surroundings? The investigators found that cells use contractile forces, generated through the small GTPase Rho, to sense structural stretch in the extracellular matrix. In a flexible matrix, cell contraction downregulates the small GTPase Rho and the focal adhesion kinase (FAK). In a rigid matrix, Rho is upregulated and FAK is enlisted to help clamp down the cells at matrix adhesion sites (inset; FAK in red, nuclei in blue). In addition to regulating cell stickiness, FAK also links to pathways that regulate proliferation and migration.

In the breast, epithelial cells must contend with numerous inputs. But in some women, cells face particularly dense breast tissue and high deposition of collagen. These women have a four- to six-fold increased risk of developing breast cancer.

Charlotte Schubert