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HIV and CXCR4 in a kiss of autophagic death

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AIDS is characterized by CD4$^+$ T lymphocyte depletion, yet the mechanisms underlying this central aspect of HIV pathogenesis are still poorly understood. In this issue of the *JCI*, Espert et al. identify a mechanism by which the HIV envelope glycoprotein can induce death in uninfected CD4$^+$ T cells (see the related article beginning on page 2161). The HIV envelope glycoprotein interacts with CXC chemokine receptor 4 to activate the lysosomal degradation pathway of autophagy, which is necessary for both apoptotic and nonapoptotic cell death.

Since the beginning of the AIDS epidemic in the 1980s, even before the HIV virus was identified, physicians and scientists recognized that a cardinal feature of AIDS was the depletion of CD4$^+$ T lymphocytes. Yet nearly a quarter of a century later, our understanding of how CD4$^+$ T cells are depleted in HIV-infected patients remains incomplete. CD4$^+$ T cells are killed by direct HIV infection, but substantial numbers of uninfected CD4$^+$ T cells also die in HIV-infected patients. The death of these cells is postulated to result from Fas-mediated activation–induced cell death and/or the stimulation of apoptosis in uninfected bystander cells by released or cell surface–expressed HIV gene products including accessory proteins (e.g., Tat, Vpr, Vpu, and Nef) and envelope proteins (reviewed in refs. 1–3).

In this issue of the *JCI*, Espert et al. describe an advance in understanding how the HIV envelope glycoprotein can kill uninfected CD4$^+$ T lymphocytes (4). By coculturing effector cells that express the HIV envelope glycoprotein with target cells that express CD4 and CXC chemokine receptor 4 (CXCR4), they demonstrate that CXCR4 engagement by the HIV envelope glycoprotein activates a lysosomal degradation pathway known as autophagy. Based on studies with pharmacological and genetic autophagy inhibitors, this activation of autophagy appears necessary both for caspase-dependent, apoptotic death of bystander cells and for caspase-independent, nonapoptotic death, which is presumably directly due to autophagy (Figure 1).

The word *autophagy* is derived from Greek and means to eat (*phagy*) oneself (*auto*). It is an evolutionarily conserved process involving the dynamic rearrangement of subcellular membranes to sequester cytoplasm and organelles for delivery to the lysosome, where the sequestered cargo is degraded and recycled. Autophagy occurs at basal levels in most tissues and contributes to the routine turnover of cytoplasmic components, playing a housekeeping function that is believed to delay aging, protect against neurodegeneration, and potentially function in tumor suppression (5–7). Autophagy is rapidly upregulated in response to different forms of cellular stress. This induction of autophagy may help promote cell survival, either by purging the cell of damaged organelles, toxic metabolites, and intracellular pathogens or by generating the intracellular building blocks required to maintain vital functions during nutrient-limiting conditions (reviewed in ref. 8). However, when very high levels of autophagy are induced, autophagy may also promote cell death through excessive self-digestion and degradation of essential cellular constituents.

There is increasing evidence that complex interrelationships exist between autophagy and the apoptotic cell death pathway (reviewed in ref. 8). Several regulators of apoptosis activation also function as regulators of autophagy activation (e.g., TRAIL, FADD, DAPk, ceramide, class I PI3K/Akt signaling, and Bcl-2 family members). Previously, it has been shown that genetic inhibition of autophagy can activate apoptotic death in nutrient-starved mammalian cells (9), suggesting that autophagy activation can function to prevent apoptosis. Conversely, it has also been suggested that autophagy activation may lead to apoptosis (reviewed in ref. 8); this conclusion was supported by data using a pharmacological inhibitor of autophagy, 3-methyladenine (3-MA), a nucleotide derivative that blocks class III PI3K activity. However, 3-MA can inhibit kinases other than class III PI3K (10), some of which may independently affect death signaling as well as inhibit the mitochondrial permeability transition (11). Now Espert et al. demonstrate that short interfering RNAs specific for 2 different autophagy execution genes (*beclin 1* and *atg7*) can completely

Nonstandard abbreviations used: CCR5, CC chemokine receptor 5; CXCR4, CXC chemokine receptor 4; 3-MA, 3-methyladenine; SDF-1, stromal cell–derived factor 1.

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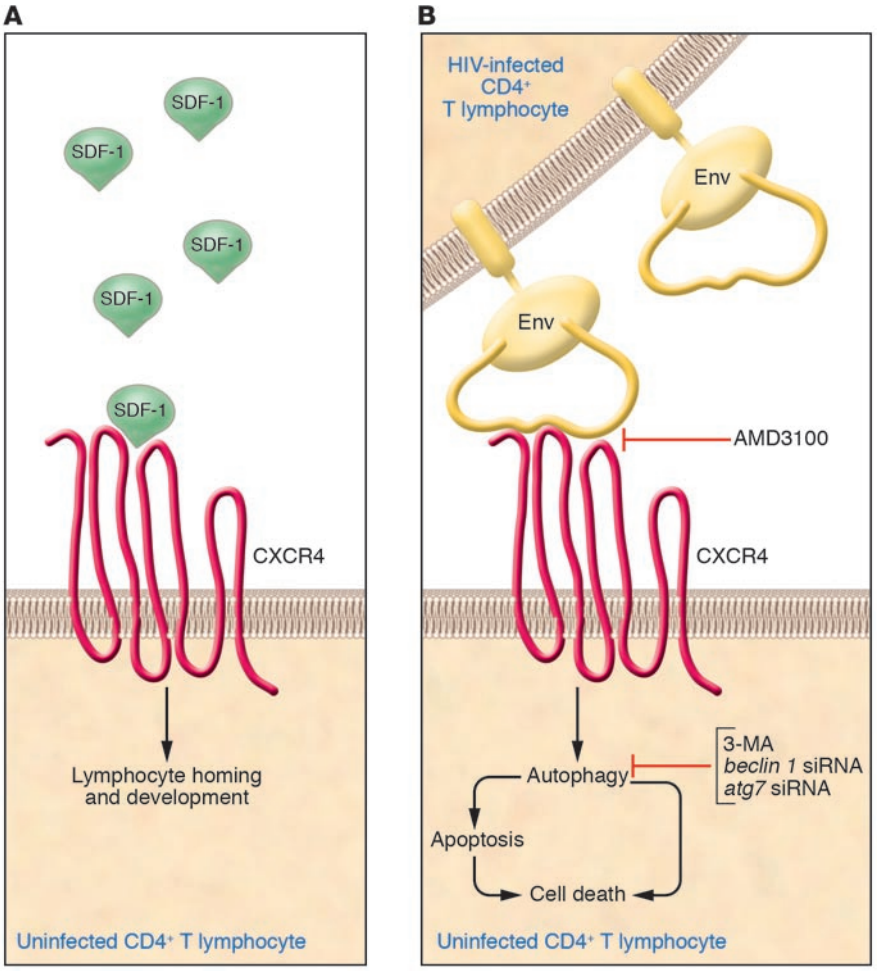

Figure 1
Model depicting events following binding of natural and viral ligands to the CXCR4 chemokine receptor. (**A**) Binding of the natural ligand SDF-1 to CXCR4 induces lymphocyte homing and functions in lymphocyte development (reviewed in ref. 17). (**B**) Binding of the HIV envelope glycoprotein (Env) to CXCR4 activates cellular autophagy, which then leads to both apoptotic and autophagic cell death. Autophagy activation and both forms of cell death are blocked by (a) a small molecule inhibitor of HIV entry via the CXCR4 receptor, AMD3100; (b) the pharmacological inhibitor of autophagy, 3-MA; and (c) siRNA directed against 2 autophagy genes, *beclin 1* and *atg7* (see ref. 4). The mechanism by which the different binding events at the same receptor in **A** and **B** result in distinct cellular outcomes is not known.

block the apoptotic death process triggered by CXCR4 engagement by the HIV envelope glycoprotein (4). This result provides genetic proof that autophagy can lie upstream of apoptosis and has implications not only for understanding how the HIV envelope glycoprotein induces bystander cell death but also, more broadly, for how the autophagy pathway interfaces with apoptosis.

Previous work has demonstrated that autophagy can be activated in virally infected cells, and that such activation requires the IFN-inducible protein kinase R signaling pathway (reviewed in ref. 12). In plants, expression of the tobacco mosaic virus p50 replicase protein is sufficient to trigger activation of autophagy both in cells expressing the protein and in neighboring cells that lack p50 expression, demonstrating that intracellular expression of a viral protein can trigger autophagy activation in bystander cells (13). Now, Espert et al. demonstrate that a viral protein expressed on the cell surface can trigger autophagy and that a viral protein can trigger autophagy by engagement of a specific cell surface receptor on neighboring cells (4). The outcome of this event, cell death, is distinct from previously described effects of autophagy in the context of other viral infections. Certain viruses (e.g., poliovirus and murine hepatitis virus) utilize components of the autophagic machinery to foster their own replication (reviewed in ref. 14). Other viruses, such as Sindbis virus and a mutant strain of herpes simplex virus that lacks an autophagy-inhibitory gene, are degraded by autophagosomes; in such cases, autophagy decreases viral replication and prevents cell death in the mouse nervous system (reviewed in ref. 12). Moreover, during tobacco mosaic virus infection in plants, autophagy activation in uninfected cells prevents rather than contributes to cell death (13).

Unlike these scenarios in which autophagy activation is protective against cell death during virus infection, the observations of Espert et al. (4) suggest that autophagy activation may also play a detrimental role for the host, as effector cells expressing the HIV CXCR4-specific envelope protein induced autophagy and cell death in uninfected, CXCR4-expressing CD4$^+$ T cells. However, it is not yet known whether a similar phenomenon occurs in the context of HIV infection in vivo and contributes to the progressive CD4$^+$ T cell depletion that occurs in patients with AIDS. If this phenomenon does occur in vivo, it could potentially explain the more rapid decline in CD4$^+$ T cell counts that generally occurs in patients with CXCR4-utilizing HIV variants (15). Yet CXCR4-utilizing HIV variants only appear in approximately 50% of the HIV-infected patients that develop AIDS (reviewed in ref. 16), suggesting that CXCR4-independent mechanisms must also exist for bystander CD4$^+$ T cell killing. The other major chemokine receptor used as a coreceptor for HIV entry, CC chemokine receptor 5 (CCR5), has also been shown to be involved in mediating apoptosis triggered by CCR5-specific HIV envelope glycoproteins, although it is unclear whether this is an important mechanism for bystander CD4$^+$ T cell death during infection with CCR5-specific viruses (reviewed in ref. 2). Thus, it will be of interest to determine whether engagement of CCR5 or other chemokine receptors by HIV envelope glycoproteins also trigger autophagy-dependent cell death in CD4$^+$ T cells.

The activation of autophagy by HIV envelope glycoprotein engagement of CXCR4 may have important implications for understanding how viral glycoproteins subvert the normal host immune response. For example, the natural ligand for the CXCR4 receptor is stromal cell–derived factor 1 (SDF-1), and this chemokine normally functions to induce migration of CXCR4-expressing T cells to

lymphoid tissues, not to induce autophagy (reviewed in ref. 17). Indeed, Espert et al. noted that SDF-1 was unable to trigger autophagy in their assays (4), indicating that the binding of HIV envelope glycoproteins to CXCR4 induces distinct intracellular signaling events compared with the natural ligand (Figure 1). These findings raise the possibility that viral proteins may selectively transduce signals through chemokine receptors that redirect the target cell away from its normal function and toward a cell suicide program driven by autophagy activation.

Conclusion

Espert et al. (4) report a very intriguing observation, namely that the HIV envelope glycoprotein induces CXCR4-dependent autophagy of uninfected lymphocytes, which is required for both caspase-dependent, apoptotic cell death and caspase-independent, nonapoptotic cell death. This work eloquently establishes that autophagy can function upstream of apoptosis in cell death signaling, that a viral envelope glycoprotein can trigger autophagy-dependent bystander cell death by binding to a cell surface receptor, and that a chemokine receptor can activate an autophagy-dependent death program in response to engagement by a viral protein. Future studies will be needed to determine whether the HIV envelope glycoprotein mediates autophagy-dependent cell death in bystander CXCR4-expressing $CD4^+$ lymphocytes in HIV-infected patients and the relative contribution of this process to the progressive decline in numbers of $CD4^+$ T cells in patients with AIDS.

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Costimulation couture: a designer approach to regulating autoimmunity

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Negative or inhibitory costimulatory pathways regulate T cell activation and play a role in peripheral tolerance. Targeting these pathways harnesses the physiologic mechanisms of regulating autoimmunity and could prove beneficial for the therapy of autoimmune diseases. However, attempts at targeting these pathways have been fraught with difficulties. In this issue of the *JCI*, Fife et al. describe a creative approach for targeting CTL-associated antigen 4 (CTLA-4) on activated T cells via genetically engineered B cells to prevent autoimmune diabetes in the NOD mouse (see the related article beginning on page 2252). Novel "designer" strategies targeting negative costimulatory pathways provide reasons for optimism in the search for a cure for devastating autoimmune diseases.

Nonstandard abbreviations used: CTLA-4, CTL-associated antigen 4; PD-1, programmed death-1; PD-L1, PD-1 ligand; scFv, single-chain, membrane-bound anti–CTLA-4 antibody.

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Evolution of the concept of costimulation: positive and negative costimulatory signals

In the early 1970s, Bretscher and Cohn proposed the 2-signal model for lymphocyte, specifically B cell, activation (1). Lafferty and colleagues later extended this model to T cell activation (2, 3). Realization that efficient T cell activation requires 2 signals (first, signal 1, an antigen-specific signal mediated via the TCR; second, signal 2, a noncognate costimulatory signal) led to the search for the costimulatory signal and identification of the CD28-B7 pathway in the early 1990s (4, 5).

Soon after the discovery of the CD28-B7 positive costimulatory pathway, it became apparent that CTL-associated antigen 4 (CTLA-4), a second inducible receptor that is homologous to CD28 and binds with higher affinity to B7-1 and B7-2, could function as a negative regulator of T cell activation (6, 7). CTLA-4 is also constitutively expressed on Tregs (8) and is important for their function (9–11) and generation (12, 13) while CD28 signaling is critical for Treg homeo-