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specific lipid compositions, starting with the outer layer, to fuse efficiently with the endosomal membrane, resulting in cytosolic import following pinocytosis. Removal of the outer vesicle then reveals two additional liposomes, which include the octa-arginine residues that help target the vesicle to the mitochondrion. Fusion occurs with both the outer and inner membranes, resulting in the final deposition of the core cargo into the matrix. In these latest experiments, the authors used DNase 1 as the cargo, with the intended proof of matrix import being the degradation of endogenous mtDNA and concomitant loss of mtDNA gene products, components of the mitochondrial OXPHOS machinery.

The production of such tailored multilamellar molecules is clearly an impressive display of nanotechnology. Many of the data compel one to believe that the vesicles have indeed delivered DNase 1 into the mitochondrial matrix. For example, the authors measured a depletion of mtDNA during the time course of the experiment. However, several observations remain unexplained. Why do the cells die while the steady-state levels of mtDNA-encoded OXPHOS components are unaffected? The toxicity of these current nanocarriers is a major concern. The authors attempted to measure the mitochondrial toxicity of these compounds directly using a well-known method based on the reduction of tetrazolium salts by dehydrogenases. Many life scientists would be familiar with this method, which uses the activity of mainly extramitochondrial dehydrogenases as an indicator of cell number.[12] It is therefore unclear why the authors claim that this assay specifically measures mitochondrial dehydrogenase or serves as a proxy for "mitochondrial function." It is possible that the authors were measuring a more general cell dysfunction and perhaps cytotoxicity directly. One possible concern is that the DNase 1 itself is being delivered not only to the mitochondrion but also to the nucleus, leading to cell death. The simple way to refute this possibility would be to perform similar experiments in cells lacking mtDNA (rho0 cells), as it is difficult to see why delivering DNase 1 to rho0 mitochondria should be toxic to cells. A second possibility is that the fusogenic liposome inadvertently perturbs the mitochondrial infrastructure, leading to a form of cell death.

Even considering these major caveats, this is still a very promising methodology. There is great potential that such concerns will be resolved with time and that a robust method for the delivery of many sorts of macromolecules to the mitochondrial matrix will become available. It is exciting to speculate that iterations of this method may eventually produce the first technique for transfecting mammalian mitochondria *in situ*.

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IL-17A in LCH: Systemic Biomarker, Local Factor, or None of the Above?

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Langerhans cell histiocytosis (LCH) is an enigmatic disease characterized by the infiltration of monocytes, macrophages, and dendritic cells, including Langerhans cells, into affected tissues. These immune cells traffic aberrantly, leaving inflammatory sequelae, organ dysfunction, endocrine deficits, and in rare cases a devastating neurodegenerative process in their wake. There remains much to learn about LCH, as debate persists as to whether it is a reactive disease of deregulated immune function or a bona fide neoplastic process; moreover, a molecular understanding of its pathobiology is lacking. Therefore, the finding from the Delprat group that interleukin (IL)-17A is elevated in the serum of LCH patients and may be involved in its pathogenesis was very welcome.[1] However, in this issue, Peters *et al.* report that they have been unable to validate this finding, having failed to identify IL-17A at either the RNA or the protein level in a large number of LCH lesions.[2] Just as independent validation of a novel biological finding is of principal importance in its garnering acceptance, so too should we value conflicting data and try to understand the discrepancies at hand.

LCH is a rare disease, affecting only some five individuals per million. The most commonly involved sites are the skin

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and bones, although LCH can also affect the lungs, liver, spleen, and bone marrow in a pattern with a higher risk of mortality. These protean manifestations have led to numerous disease eponyms for distinct clinical patterns; Hand-Schüller-Christian and Letterer-Siwe disease are just two examples of many. Fortunately, these are all now reunited under the LCH moniker because they share common histopathological features. Although LCH is not considered by most to be a malignant process, the discovery of oncogenic *BRAF* mutations in diverse LCH lesions has reinvigorated the camp arguing for a neoplastic origin.[3] This finding awaits validation, but it is intriguing because it allows speculation that a subset of LCH reflects a premalignant inflammatory process driven by aberrant *BRAF* signaling, whereas progressive refractory LCH might reflect additional cooperating lesions leading to a more oncogenic phenotype, analogous to the benign nevus-to-melanoma continuum in which *BRAF* participates.

The predominant therapies employed for LCH reinforce this immune dysregulation/neoplasia dichotomy, given that children with limited disease benefit from modest immunosuppressive therapy whereas those with more aggressive forms typically require conventional chemotherapy. However, in an era of expanding molecularly targeted therapies for inflammatory disease and cancer, it is disappointing that more refined approaches have yet to be identified. This is a consequence of our limited knowledge of the underlying biology. It also explains the high level of interest in the Delprat group's finding that dendritic cell (DC)–secreted IL-17A was a potential driver of LCH-associated pathology. In an elegant series of experiments following their identification of increased levels of IL-17A in the serum of LCH patients, the authors demonstrated that recombinant *IL-17A* induced monocyte-derived DCs to adopt a mixed macrophage–DC phenotype with the subsequent formation of multinucleated giant cells through a novel cell fusion pathway. DCs from LCH patients demonstrated a similar phenotype in the absence of exogenous IL-17A, supporting the notion that they secreted functional IL-17A and adopted the multinucleated giant cell form typical of LCH granulomas. Furthermore, although total serum IL-17A did not correlate with disease activity in the LCH cohort, a serum-based cell fusion bioassay did, presumably by assessing functional IL-17A activity. Collectively this work identified a novel pathway that helps to explain many of the pathological features of LCH lesions while also providing a possible biomarker with which to monitor disease activity.

Into this background comes the report from Allen and colleagues that they were unable to identify *IL-17A* RNA or protein in a large number of LCH lesions and flow-sorted candidate cell types suspected of secreting this cytokine (including $CD207^-$, $CD1a^+$ cells).[2] Notably, the Delprat group focused only on the detection of IL-17A protein without mRNA-based studies, and Allen and McClain previously reported their inability to identify *IL-17A* mRNA in flow-sorted T cells ($CD3^+$) or LCs ($CD207^+$) (ref. 4). Here they extend this to additional samples and cell subtypes, using quantitative PCR methodology with three different primer/probe sets, reverse-transcription PCR with primers for each exon, and Affymetrix gene-chip probe-set data. Importantly, they also assessed protein expression using commercial polyclonal and monoclonal anti-IL-17A antibodies. Their findings, based on a principal antibody of the prior study, support an unfortunate cross-reactivity with a protein that migrates with the same approximate molecular weight as IL-17A (ref. 1). Mass spectroscopy techniques (liquid chromatography–tandem mass spectrometry with an LTQ Orbitrap) failed to identify any IL-17A peptides from this band, and the antibody affinity for this protein was weak.

What, then, does one make of such conflicting reports? The data reported here are rather compelling, and it is difficult to imagine sufficient systemic IL-17A secreted by LCH patients' DCs in the absence of any detectable message for this gene in the lesions themselves. The absence of a correlation between plasma or serum IL-17A levels and disease activity from either group, and the current demonstration that the enzyme-linked immunosorbent assay antibody used has specificity concerns, make it quite unlikely that IL-17A can serve as a useful systemic biomarker. But could IL-17A be produced locally to act in an autocrine or paracrine fashion on cells within LCH lesions to drive the noted disease-associated attributes? Again, the lack of identifiable IL-17A transcripts or validated protein in the lesions suggests that this is not likely. The risk here, however, is that we will lose an opportunity to understand an important facet of DC and LC pathobiology. Indeed, much of the Delprat group's work using recombinant *IL-17A* to define a novel cell fusion pathway is free of the antibody-specificity controversy and remains of great interest. They also recognized that some patients who did not have detectable serum IL-17A did have detectable anti-IL-17A-blockable fusion activity in their bioassays. The investigators proposed that this resulted from an "IL-17A–like molecule." It remains plausible that some of their observations related to IL-17A activities in DCs, as well as the phenocopying of LCH disease attributes, might also be induced by an alternative cytokine. The IL-17 family has a large membership; might one of these members or another cytokine therefore be a candidate for this factor?

These issues are of more than academic interest, given that therapeutics that target IL-17A are now in preclinical development internationally for use in LCH and other inflammatory conditions. The histiocytosis research community is a robust one that has made strong inroads into understanding the genetics of histiocytic disease and improved patient outcomes through a series of international clinical trials. Here, then, is an opportunity to add to our collective knowledge of LCH by working together to understand the IL-17A controversy and to ensure that novel therapeutics brought to testing in the clinic for this rare disease are rational and biologically driven. To quote Benjamin Rush: "Controversy is only dreaded by the advocates of error."

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