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INSIDE LAB INVEST

ESCAPE FROM THE ER: APICAL CFTR IN CYSTIC FIBROSIS: A decade has passed since the identification of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, and its encoded membrane protein, a cAMP-activated chloride channel. Most CF patients harbor a trinucleotide deletion in CFTR, causing the loss of a phenylalanine at position 508 (F508del). This mutation results in failed CFTR function, with consequential underhydration of mucous in the respiratory and GI systems and a host of secondary pathologic changes. Less clear has been how this single amino acid deletion alters CFTR function. Soon after the discovery of CFTR, studies of transfected cells in vitro established that the newly synthesized mutant (F508del) CFTR protein had difficulty exiting the endoplasmic reticulum, and instead was shunted for degradation early in the secretory pathway. This led to the concept of CFTR as an archetypal ER-retention disorder, a notion bolstered by observations that at experimentally low temperature, mutant CFTR could reach the plasma membrane and would function there. Yet, as work has progressed, it has become clear that most CFTR, even the wild-type, is degraded after synthesis, and that the differences between the proportion of mutant versus wild-type CFTR exiting the ER, while real, are not as great as first thought. In addition, and in contrast to the recombinant systems studied experimentally, biochemical evidence has begun to emerge that at least a fraction of the mutant CFTR does indeed leave the ER. The implications of these findings, if true, are significant because they imply that mechanisms other than just the retention of putatively misfolded protein in the ER may be at play, and broaden the range of theoretical targets for therapeutic intervention. This month's contribution by **Penque and her colleagues** (Lab Invest 2000;80:857–868) adds important new observations to this conundrum. By comparing the CFTR distributions in freshly obtained respiratory epithelial cells from patients homozygous or heterozygous for (F508del) CFTR with normal patients, artifacts arising from in vitro systems using recombinant proteins are ruled out. Their results are clear: apical pools of CFTR, distinct from the ER or Golgi, are present even in homozygous cystic fibrosis patients. While the number of cells with apical CFTR is greater in normal patients, consistent with a trafficking defect, these results suggest that the defect might act in not only ER retention and degradation, but also perhaps the recruitment of a sub-apical pool of CFTR to the plasma membrane. Alternative possibilities consistent with these findings include a role for decreased membrane stability or retention of CFTR. In either event, these results add important new perspectives to our understanding of this tragic disorder, and strengthen the likelihood that novel approaches that enhance the activity or display of even mutant CFTR may exist.

WHAT TO EXPECT IN XENOTRANSPLANTATION: It is highly likely that clinical experimentation involving the transplantation of pig kidneys and hearts into human recipients will begin within the next year or so. If successful, such transplantation of xenogeneic (ie, non-human) organs into humans (commonly referred to as "xenotransplantation") could revolutionize medicine by solving the severe human organ shortage that has limited the availability of allogeneic (human-into-human) transplantation, a highly (and perhaps the only) effective treatment for end stage organ failure. What problems are likely to be encountered? A theoretical concern is transmission of infectious diseases (especially pig endogenous retroviruses) from pig to human, and this will be closely monitored. A more immediate practical difficulty will be immunological rejection. Antibody-mediated rejection is a particular problem for xenografts from distantly related (discordant) species, such as the pig into human species combination, and this will need to be suppressed (eg, by using genetically engineered pigs that resist complement-mediated damage) to prevent immediate (hyperacute) graft loss. Cell-mediated rejection is the principal immunological barrier in allogeneic transplantation, and it is unknown how significant this response will be for xenografts. Cell-mediated recognition of allografts can be divided into two components. The indirect component involves the host immune system treating the graft as any other foreign antigen, processing graft proteins as if they were microbial proteins. Xenograft proteins will differ more strongly from the human host than do allograft proteins, and the indirect component of the cellular immune response will likely be stronger against pig xenografts than human allografts. However, the major cell-mediated response in allografts arise from the direct component, which involves T cell recognition of intact graft cells as foreign. Direct recognition arises from host T

cell receptor binding to intact non-self MHC molecules expressed on graft antigen-presenting cells (APC). T cell activation by host or graft APC also requires signals from antigen-independent costimulator molecules and cytokines as well binding through adhesion molecules that are expressed by the APC. In the case of xenografts, a sufficient divergence in the structure of any of these types of molecules can reduce or even prevent direct recognition of graft cells, weakening the immune response. The extent of conservation of these interactions is a variable and depends upon which two species are involved. For this reason, rodent xenotransplant models may be of limited utility in predicting the events that will be observed in human recipients of pig organs. Pig organ transplantation into old world primates is perhaps the best predictive model available of what is in store for patients. Last year, a group of investigators from the Massachusetts General Hospital described a pig to primate xenotransplantation protocol that successfully overcame hyperacute rejection (by depletion of anti-pig antibody) coupled with an (unsuccessful) attempt to induce immune tolerance to pig antigens. These organs succumbed to a rejection response that developed over several days, previously termed delayed xenograft rejection (DXR) because it is slower than hyperacute rejection. DXR in rodents has been described as a response mediated by host NK cells and cytokines. This may be a default pathway in species combinations in which direct recognition of xenogeneic cells by host T cells is defective. In this issue **Shimizu and colleagues** describe in detail the pathological findings that characterize the form of DXR that develops against pig organs in the primate (Lab Invest 2000;80:815–835). Remarkably, these findings differ profoundly from the previously studied rodent models and look much more like the response of a presensitized human host against an allograft, sometimes called accelerated rejection. The principal features of primate-versus-pig DXR in the kidney are antibody and complement-mediated endothelial injury in glomeruli and peritubular capillaries. Progressive antibody and complement deposition was accompanied by endothelial injury and by accumulation of host T cells, including cytolytic T lymphocytes, the principal effector cell of acute allograft rejection. The involvement of T cells may be explained by the preservation in primates (and humans) of direct T cell recognition of pig cells. NK cells, far from being a prominent component, were essentially absent from these lesions. The authors propose that the general term DXR be replaced by the more specific descriptor “acute humoral xenograft rejection” to emphasize the central role of antibody in this process. Two important conclusions may be drawn from this pioneering study. First, antibody and T cells rather than NK cells are likely to be the principal targets for suppressing human anti-pig xenograft rejection once hyperacute rejection is controlled. Second, immunopathologists are positioned to play a key role in understanding clinical xenotransplantation just as they did in elucidating the mechanisms of rejection in clinical allotransplantation three decades ago.

VASCULAR APOPTOSIS AND TUMOR ANGIOGENESIS: ONE STEP BACKWARD—TWO STEPS FORWARD? The development of a tumor vasculature is necessary for continued tumor growth and also facilitates metastatic spread. Tumor angiogenesis has been associated with the secretion of angiogenic factors by tumor cells, and inhibition of angiogenic factor-driven tumor angiogenesis has been accomplished using antibodies directed against selected angiogenic factors and soluble angiogenic factor receptors. In this issue of the journal **Zagzag et al** propose a novel alternative mechanism of tumor angiogenesis operational in normally highly vascularized tissues/organs such as brain and lung (Lab Invest 2000;80:837–850). They postulate that in certain circumstances tumor cells can transiently subvert the normal tissue microvasculature by homing to it and utilizing it transiently for nutrient/waste exchange. Using a murine model of glioma implantation in the brain, they illustrate an early “homing” of tumor cells to perivascular areas, associated with a concomitant lifting and replacement of astrocytic endfeet with tumor cells. Over time, the investigators noted Ang2 expression and a vascular involution followed by tumor necrosis. They postulate that it is this later necrosis and hypoxia that then triggers tumor angiogenesis and subsequent tumor growth. This temporally-staged two phase vascular response during glioma growth in the mouse may provide us with a model system to better examine and elucidate dynamic tumor cell-vessel interactions as well as system in which selected soluble pro- and anti-angiogenic factors may be examined in an in vivo context.

JUST IN TIME FOR HILLARY: CGH AND THE NEW YORK STATE SENATE RACE: Will mayor Giuliani run or will he withdraw from the race for the New York State Senate seat? After a press conference and a front page New York Times report announcing that the mayor of New York City and Republican candidate for the Senate seat

has prostatic cancer the question is being asked in both campaign headquarters. And wouldn't Ms. Clinton like to know! It turns out that a relatively simple study of the mayor's cancer is likely to produce the answer. If the prostatic cancer cells have not gained chromosomal material located in the 7pq and/or 8q regions, the tumor is not likely to progress whereas gains in both of these regions predict an aggressive behavior. The studies supporting this assertion appear in this issue of the journal (Lab Invest 2000;80:931–942). **Alers and co-workers** have conducted CGH studies of primary and metastatic cancers and have identified chromosomal alterations that appear to serve as reliable markers of the biological potential of a prostatic adenocarcinoma. Although the number of cases studied is substantial, this interesting report will need confirmation before important decisions are based on the findings presented by Alers et al. But the study clearly demonstrates the potential of the technologies that enable a comprehensive analysis of cancer at the molecular level. Comprehensive and systematic study of well-characterized cohorts of patients is likely to yield novel and practical information. The pathologists that have for years accurately and reliably identified cancer will be able to refine tumor classification adding a molecular nosology. This taxonomy is likely to be predictive of the tumor behavior when conventional histology was not resolutive and should allow for a more specific selection of therapeutic drugs. Knowledge of the DNA complement of certain cancer cells could, in this instance, help to make what would be called a very educated guess!