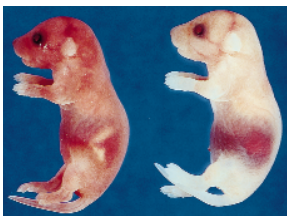
In this issue

By John Ashkenas, Science Editor

Early developmental roles for clotting factors

(See article on pages 1547–1554.)

Blood clotting in response to vascular injury is crucial in postnatal life but apparently dispensable in fetal development. Nevertheless, as Parry and Mackman note, knockouts in several genes associated with this pathway — those for prothrombin, Factor (F)V, and tissue factor (TF), among others — cause mice to die in mid-gestation with vascular abnormalities. The occasional survivors may live through the gestation period, depending on strain background, but are prone to severe hemorrhaging during or shortly after birth. TF, a transmembrane protein expressed on perivascular cells, binds through its extracellular domain to FVII and FVIIIa, to generate an active proteolytic complex, while its short intracellular domain contacts the actin cytoskeleton. Parry and Mackman previously reported that low levels of TF expression, provided by a human transgene, are adequate to see the embryo through the period of early vascular development and can even permit postnatal survival, although death from spontaneous hemorrhages often occurs in the early weeks of life. Here they show that the early, nonhemostatic role of TF can be rescued even in the absence of its cytoplasmic domain, suggesting that signaling or cellular shape change mediated by this receptor is not required for vasculogenesis. The extracellular portion of the molecule, however, is essential at this stage, and a mutant form of TF that fails to bind FVII and FVIIa fails to rescue the defect. The authors speculate that the TF complex participates with prothrombin in a proteolytic cascade that helps maintain vessels during development.



Successful delivery of a transgene across the apical surface of the lung epithelium

(See article on pages 1573–1587.)

Like other epithelia, bronchioles express different proteins and carry out distinct functions on their basolateral and apical sides. In culture models that preserve epithelial polarity, transgene delivery is far more efficient when attempted from the normally inaccessible basolateral side than the apical side. The persistent failure of transgene delivery to the airway through inhalation of recombinant vectors has been plausibly explained by the absence of viral receptors from the apical surface of airway cells, but Duan et al. now show that, for adeno-associated virus-2 (AAV-2) vectors, the barrier to expression of apically delivered transgenes occurs after viral uptake. Whereas basolaterally endocytosed virus undergoes processing in endosomes and traffics efficiently to the nucleus, the small amount of AAV-2 that is internalized from the apical surface is blocked in its processing and rarely reaches the nucleus. Remarkably, inhibitors of ubiquitination or proteasomal degradation greatly increase the efficiency of viral trafficking and maturation steps, for both basolaterally and apically internalized virus. In vivo treatment with a proteasome inhibitor increases the efficiency of viral transduction by inhaled recombinant AAV-2 from undetectable levels to about 10%, at least in larger bronchioles. The nature of the apical uptake pathway, which may be receptor-independent, remains to be identified, as does the relevant target of proteasomal degradation. Duan et al. show that viral proteins are ubiquitinated, but indirect effects on viral trafficking may also be at work, perhaps regulated by an intrinsically unstable cytoplasmic protein.

Mechanisms of T-cell help in anti-tumor immunity

(See article on pages 1623–1630.)

$CD4^+$ T cells are required not only to induce humoral immunity, but also to activate the cytotoxic effects of $CD8^+$ T cells, and in many tumor vaccination strategies the latter interaction is crucial. Lode and coworkers show here that one such strategy using an immunocytokine — a chimeric molecule containing IL-2, which is directed to tumor cells through a specific antibody domain — depends primarily on $CD8^+$ cell responses for its effect against metastatic disease. $CD4^+$ T cells also contribute to the response, since immunodepletion of these cells allows metastatic foci to proliferate to some extent. The authors use mice with defined genetic defects to distinguish between two mechanisms by which $CD4^+$ T cells might activate $CD8^+$ T cell–dependent cell killing: by inducing endogenous IL-2 synthesis, or by activating dendritic cell surface CD40 protein with the $CD4^+$ T cell–borne CD40 ligand (CD40L). Lode et al. show that knockout animals that cannot synthesize IL-2 still suppress tumor growth. Hence, the former mechanism is clearly not required for the effect of the immunocytokine, perhaps because the treatment already provides maximal levels of IL-2 stimulation. On the other hand, the CD40/CD40L interaction appears to be important, since the effect of the treatment is blunted in CD40L-deficient animals but can be restored by supplying an activating antibody to CD40 in place of the endogenous ligand.