Emerging novel functions of neutrophils

Neutrophils were first described in the 1800s and crucial protective functions as short-lived killer cells against pathogens have been characterized; however, they may also cause excessive tissue damage. In this Invited Review, Paul Kubes and colleagues update us about the many novel functions, under steady-state conditions and after tissue injury, that have been revealed by new experimental techniques. They describe tissue-resident neutrophils that survive longer than had previously been thought and acquire tissue-specific characteristics. The authors detail the importance of neutrophil clearance in the bone marrow versus periphery in regulating hematopoiesis, their potential role regulating lymphocytes in secondary lymphoid organs, their influence in regulating liver metabolism and how they prevent mucosal inflammation. Turning to the roles of neutrophils in tissue repair, the authors discuss the varying and complex situations where neutrophils may be reparative or harmful, and detail the versatile mechanisms in the liver, skin, nervous system and heart in a range of disease states and models.

A new model delineates immune responses in epidermal candidiasis

Candida albicans can harmlessly colonize epithelial surfaces but can also cause severe infections on surfaces or within tissues. The IL-17 family members IL-17A and IL-17F are crucial to prevent C. albicans infection in humans and mice. Most models use abrasion to inoculate the fungus but Iwasawa et al. use a new, non-abrasive model of superficial epicutaneous candidiasis. Yeast and hyphal forms on the skin surface induce mild inflammation, featuring cytokine production, parakeratosis and neutrophil infiltration by day 2, which reduces by day 7 in wild-type mice. IL-17A plus IL-17F (but not either alone) are essential for day-7 fungal clearance (see figure). The lesional cells that produce IL-17 are group 3 innate lymphoid cells (ILC3s) and γδ T cells but not Th17
cells or neutrophils. In the absence of various other cell types, IL-17-producing ILC3s can efficiently clear *C. albicans*, and neutrophils are essential for this; however, various innate-immune receptors that recognize *C. albicans* are not essential.

**CDR-shuffled alpaca antibodies capable of binding human proteins**

The demands to use synthetic antibody molecules are increasing and driving various approaches for industrial production. Camelid IgG2 and IgG3 have features that make them suitable for this; they lack immunoglobulin light chains and have the heavy-chain variable domains (termed VHH) consisting of three complementarity-determining regions (CDR1–CDR3). Using peripheral lymphocytes from unimmunized alpacas or those immunized with human serum albumin (HSA), Tsukahara et al. constructed a recombinant VHH phage-display library in which various gene sequences can be expressed on bacteriophages. They used CDR shuffling (see figure) to construct new combinations of the amino-terminal or carboxy-terminal halves of different VHHs. The authors analyzed the sequences of VHHs, their binding to HSA and non-immunized human proteins, and their thermal stability. Similar proportions of VHHs against non-immunized proteins and the immunized protein, HSA, suggest that this method allows rapid generation of engineered antibodies that bind diverse targets.
B4 and integrins co-tether fibronectin on macrophages

Fibronectin (FN) is an essential component of the extracellular matrix and also acts as a ligand to trigger various cell functions. FN can be tethered to cell-surface integrin α-β subunits, leading to FAK-mediated and Syk-mediated signaling pathways. Human LILRB4 (B4) and its murine ortholog gp49B are inhibitory immune-checkpoint molecules and FN binds B4/gp49B and integrins at different sites (the FN30 N-terminal 30-kDa fragment and the RGD motif). In their Short Communication, Itoi et al. examine whether B4/gp49B and integrins cooperatively tether FN on macrophages and the effects on integrin signaling. B4/gp49B, FN and integrins indeed form a triplet in close vicinity on bone marrow-derived macrophages and resident peritoneal macrophages. Even if FN is absent, gp49B and β1-integrin are in close vicinity at the focal adhesion site on glass surfaces and B4/gp49B negatively regulates the Syk-mediated pathway. Thus, B4/gp49B and integrins co-tether FN in cis on the same cell and the triplet regulates integrin-mediated signaling in macrophages.