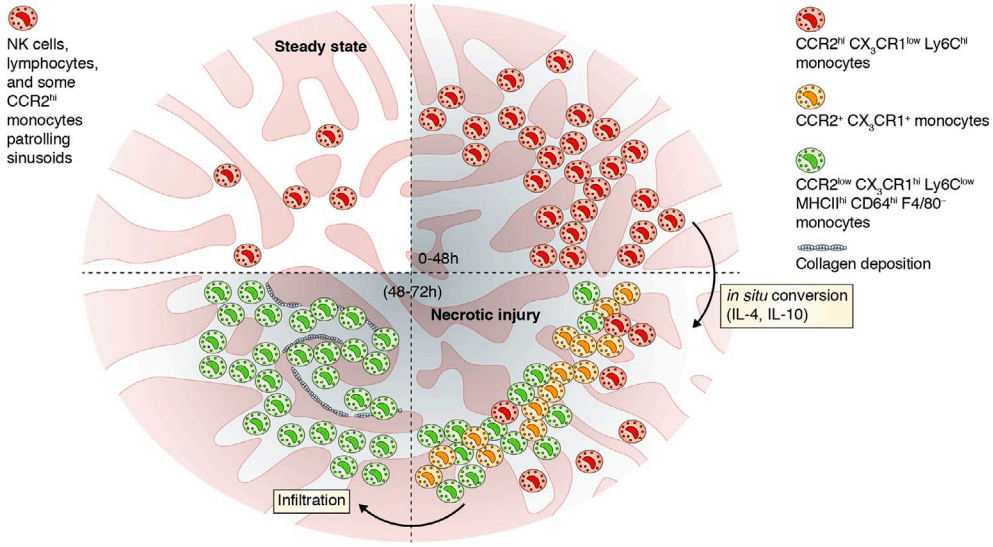
Monocyte phenotypes: When local education counts

Insight from (left to right) Amado Quintar, Catherine Hedrick, and Klaus Ley

Monocytes are a heterogeneous population of phagocytic cells that are generated in the bone marrow and released into the bloodstream. There are two main monocyte subsets in mice: "inflammatory" monocytes that are Ly6C^{hi} CCR2^{hi} CX$_3$CR1^{low} and "alternative" or "patrolling" monocytes that are Ly6C^{low} CCR2^{low} CX$_3$CR1^{hi}. The process of monocyte recruitment and differentiation is still a matter of controversy. In this issue, Dal-Secco et al. report in situ monocyte reprogramming in the liver, from proinflammatory CCR2^{hi} CX$_3$CR1^{low} cells into reparative CCR2^{low} CX$_3$CR1^{hi} cells and show, for the first time, that this occurs at the site of injury.

Dal-Secco et al. harnessed the power of imaging technology and fluorescent reporter mice to track monocytes in a model of sterile inflammation in the liver (induced after a 30 μm^3 burn by a thermal probe). In this model, the recruitment of monocytes is dependent on CCR2 present on CCR2^{hi} CX$_3$CR1^{low} monocytes. Some of these cells were observed patrolling the liver sinusoids in the steady state. Within 24–48 hours, CCR2^{hi} CX$_3$CR1^{low} monocytes extravasated and formed a ring structure surrounding the necrotic tissue at the site of the injury. These extravascular monocytes started to differentiate and, by 48–72 hours, had lost CCR2 expression and gained CX$_3$CR1 and MHC class II expression (CCR2^{low} CX$_3$CR1^{hi} CD11b^{hi} MHCII^{hi} CD64^{hi} F4/80$^-$). Blocking IL-10 and IL-4 delayed this phenotypic transition and impaired wound healing as shown by failure to clear necrotic hepatocytes and failure to deposit collagen.



A focal sterile injury induces the recruitment of CCR2^{hi} monocytes (red) to the liver, which form a ring surrounding and "walling off" the necrotic lesion. After 48–72 hours, monocytes start to gain CX$_3$CR1 expression (orange) and undergo an IL-4/IL-10–mediated phenotypic conversion into CCR2^{low} CX$_3$CR1^{hi} CD11b^{hi} MHCII^{hi} CD64^{hi} F4/80$^-$ monocytes (green). These cells infiltrate the necrotic tissue and induce collagen deposition leading to the clearance of dead cells.

These findings reveal a mechanism for monocyte transformation in situ in response to sterile inflammation in the liver, starting with the abundant pool of circulating Ly6C^{hi} CCR2^{hi} CX$_3$CR1^{low} monocytes and exploiting their plasticity for on-site education and conversion. Monocyte conversion from inflammatory to alternative phenotypes has previously been shown to occur in the bone marrow, but this paper shows that phenotypic conversion can also occur in the liver. The dependence on IL-4 and IL-10 for this phenotypic switch is reminiscent of alternatively activated macrophages, similar to wound-healing M2 cells. Further work will be required to investigate the hepatic source of IL-4 and IL-10 and determine how these cytokines induce phenotypic conversion. Investigations that address the nature and source of the CCR2 ligands that recruit inflammatory monocytes to sites of sterile injuries and of the chemokine(s) that facilitate entry of CX$_3$CR1^{hi} monocytes into necrotic lesions may also provide fascinating answers.

These results provide new data on the plasticity of monocytes in the tissue microenvironment which may lead to new opportunities for therapeutic intervention in disease.

Dal-Secco, D., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141539.

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Breaking the allergic response by disrupting antibody glycosylation

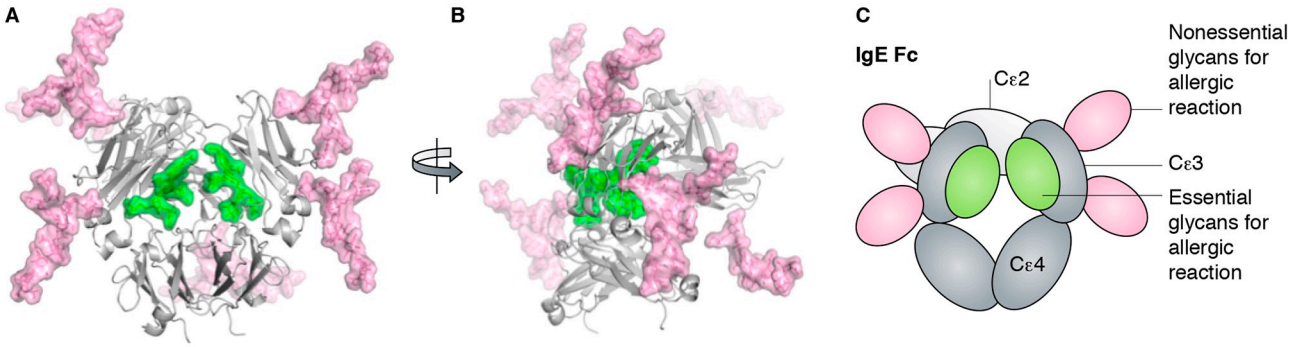
Insight from Max Crispin

Allergic reactions arise when people become sensitized to otherwise harmless environmental antigens. In this issue, Shade et al. reveal that the immunoglobulin ε (IgE) antibodies that mediate these reactions have a key vulnerability. They report that the ability of IgE to trigger an allergic reaction through its interaction with mast cells is dependent on a single site of antibody glycosylation. With the in vivo targeting of specific glycoprotein glycans emerging as a viable strategy for modulating endogenous glycoprotein function, these findings are of significant interest.

IgE is found tightly bound to mast cells through the interaction with the high-affinity Fcε receptor (FcεRI). This interaction is sufficiently tight that IgE antibodies are stably immobilized on the immune cells, sensitizing them to specific antigens for extended periods of time. The IgE–FcεRI interaction occurs through the IgE Fc domain. Glycosylation of antibody Fc domains has been known to be important in the interaction of IgG with cellular Fcγ receptors, but there has been conflicting evidence on the role of the equivalent IgE Fc glycans. Shade et al. show that glycosylation of IgE is essential for both FcεRI binding and the triggering of mast cell degranulation. Using a panel of mutants, they demonstrate that IgE effector function is dependent on the glycan at a single glycosylation site in the Cε3 domain at Asn394 and Asn384 in human and mouse IgE, respectively.

Typically mammalian glycans are efficiently processed during glycoprotein folding and secretion from the oligomannose-type glycans added cotranslationally to a highly heterogeneous mix of complex-type glycans. The Cε3 glycosylation site identified to be critical for receptor binding has been shown by Shade and others to be trapped in the oligomannose state. Examination of the previously reported crystal structure reveals that these glycans are located in the interstitial space between opposing Cε3 of the homodimeric IgE Fc. The efficient processing to complex glycans is likely to be limited by either the extensive glycan–protein interactions or by steric blocking of the processing enzymes by the wider proteinous environment. Importantly, Shade et al. suggest that the unusual presence of oligomannose glycans at this critical site offers an opportunity to specifically target this structure to shut down the allergic response. They show that a bacterial endoglycosidase from *Flavobacterium meningosepticum* (EndoF1) that specifically hydrolyses oligomannose-type glycans can disrupt IgE effector functions. Whether bacterial enzymes can ever be used clinically is a point of some debate. Despite their ability to turn off elements of the immune response, it is likely that the immunogenicity of bacterial enzymes will limit any clinical applications to a single dose. The IgG-degrading enzyme from *Streptococcus pyogenes*, IdeS, is being investigated as a single-dose treatment to enable kidney transplantation for patients who have antibodies that target prospective kidneys and, encouragingly, has recently been shown to be well tolerated in humans in a small-scale safety trial.

These clinical considerations aside, this study demonstrates the importance of IgE glycosylation in allergic responses and provides a route to the selective disruption of this mechanism in allergic diseases.



(A and B) Molecular model of the glycosylated IgE Fc domain. The IgE Fc glycans are shown as sticks with surface representation. (C) The structure of panel A is illustrated schematically and the glycans are colored throughout according to their requirement for FcεRI-mediated immune response. Molecular model courtesy of Dr. Mark Wormald.

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