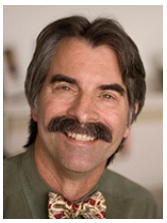
Major progress in understanding progression in chronic myeloid leukemia



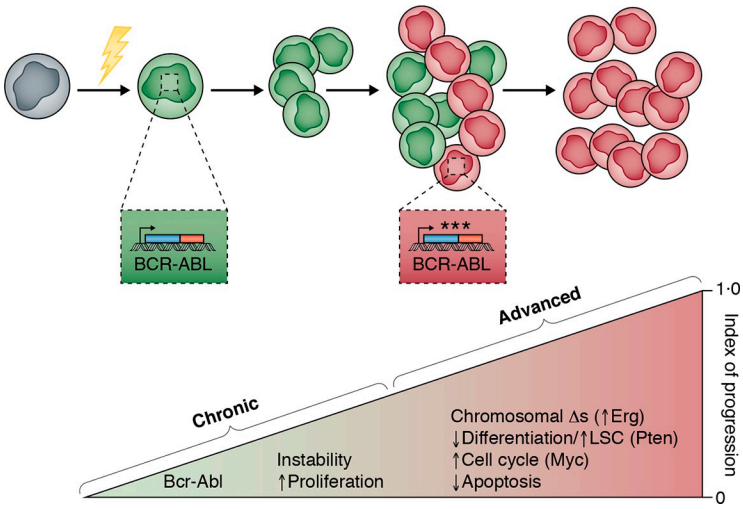
Insight from Jerry Radich

At last, a mouse model of the natural history of chronic myeloid leukemia (CML) has been elegantly engineered.

CML is hardly a public health menace, occurring in only 1–2 per 100,000 people. Nonetheless, the disease is the "poster child" of genetically based diagnosis and treatment, as all cases of CML have the BCR-ABL translocation, which is both a diagnostic marker of the disease and a target for tyrosine kinase inhibitor (TKI) therapy. CML is usually diagnosed in the so-called chronic phase, characterized by an expansion of circulating mature myeloid cells. Without treatment, all CML cases will eventually accumulate new mutational events, progressing first into accelerated phase and then to a fatal blast phase. The advent of TKI therapy has made a major impact on the natural history of chronic phase disease, and few patients now progress on therapy. However, some do, and still other patients actually present with advanced phase disease. For these patients, therapeutic options are limited and generally ineffective.

The genetic "clock" that drives CML progression is unknown, and this limits development of diagnostic tools to predict progression and therapeutic options to block or treat it. A major part of this limitation is the lack of mouse models of CML that accurately simulate human CML. Most mouse CML models quickly develop an acute leukemia, often of the lymphoid lineage (unlike CML blast crisis, which is predominantly myeloid), or stay in a chronic phase. In this issue, Giotopoulos et al. provide a major contribution to the field by developing a cleverly engineered mouse model that quite faithfully duplicates human CML.

Their mouse model has both inducible BCR-ABL and "Sleeping Beauty" transposon elements, allowing them to first activate BCR-ABL (mimicking chronic phase), and later to activate transposon-based insertional mutagenesis (mimicking progression). The model shows many features of human CML, including progression from chronic phase to a predominantly myeloid blast crisis, expansion of the hematopoetic stem cell and progenitor cell compartments, and similar changes in gene expression from chronic to blast phase as those reported in human samples (much to the relief of both mouse and human investigators!).



A highly simplified model of CML progression. A genotoxic insult (lightening bolt) cases the BCR-ABL translocation. BCR-ABL induces myeloid proliferation and also results in genomic instability. Without therapy, unopposed BCR-ABL signaling causes new chromosomal changes, mutations, and changes in gene expression, causing a block in differentiation, further increases in cell cycling, and decreased apoptosis. In the figure, * indicates the genetic changes in addition to BCR-ABL that promote progression to advanced phase CML (accelerated and blast phases). The "index of progression" is an artificial construct suggesting the cumulative affect of many changes in gene structure and function.**

The authors find a role for pathways that are potentially targetable by existing and investigational agents, including ERG, MYC, MEK, RAF, and JAK1/2. This will likely lead to the rapid development of mouse models in which to study whether such agents can either treat or prevent blast crisis. Because the therapeutic options for humans with advanced phase are severely limited (with curative potential limited to allogeneic transplantation), the findings from this paper will also likely quickly lead to the study of these pathways in patients with advanced phase disease, with possible intervention in those cases where activation can be demonstrated. The outcome for patients with blast crisis has remained relatively static for decades. The findings from this strong manuscript suggest that may soon change.

Giotopoulos, G., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141661

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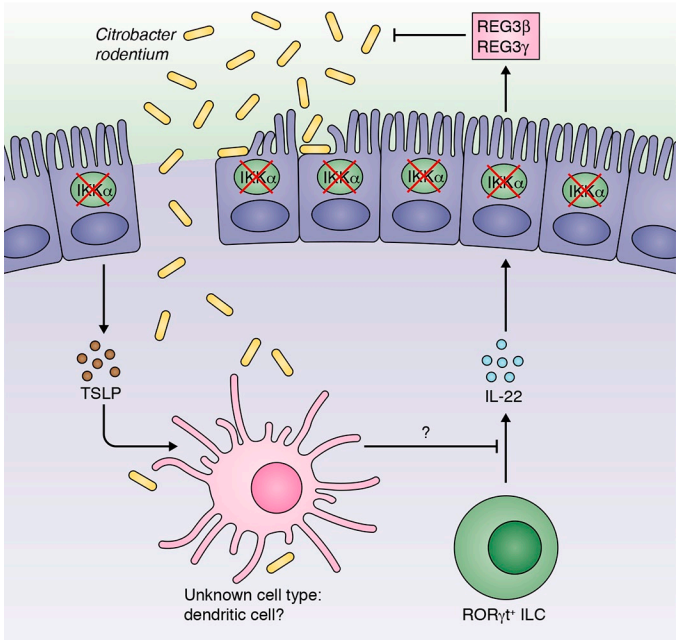
Epithelial IKKα licenses ILC3s to defend the intestinal barrier

Insight from Christina Eftychi (left) and Manolis Pasparakis (right)

In this issue, Giacomin et al. report on a novel function of IKKα acting in intestinal epithelial cells (IECs) to control intestinal immunity against enteropathogenic bacteria. Human enteropathogenic and enterohemorrhagic *E. coli* constitute a serious health concern, as they can cause life-threatening intestinal infections. Infection of mice with *Citrobacter rodentium,* a natural enteric mouse pathogen, serves as a relevant model to study the mechanisms of host immunity against this family of enteropathogenic bacteria.

Giacomin et al. found that mice with IEC-specific ablation of IKKα (IKKα$^{\Delta IEC}$), but not IKKβ, were highly susceptible to *C. rodentium* infection. A series of elegant follow-up experiments revealed that epithelial IKKα deficiency caused impaired production of IL-22, a cytokine that promotes epithelial regeneration and antimicrobial defense, by group 3 innate lymphoid cells (ILC3s) in infected mice. Administration of recombinant IL-22 or transfer of IL-22 producing ILCs restored effective immunity to *C. rodentium* in IKKα$^{\Delta IEC}$ mice. The IKKα-deficient epithelium produced increased amounts of TSLP, and treatment with neutralizing antibodies against TLSP largely restored IL-22 production and effective immunity to *C. rodentium*. Interestingly, recombinant TSLP inhibited IL-22 production by RORγt^{+} ILCs when added in splenocyte cultures but not in purified ILC cultures, suggesting that the suppressive effect of TSLP on IL-22 production in ILCs is indirect.

The study by Giacomin et al. adds novel insight to our understanding of the epithelial–immune cell dialog that controls intestinal immunity, but it also raises new questions. Does epithelial IKKα act via noncanonical NF-κB signaling, as suggested by previous studies implicating epithelial lymphotoxin β receptor and stromal p52 in controlling *C. rodentium* infection, or do other, NF-κB–independent, IKKα functions regulate intestinal immunity? Additional studies in mice with epithelial cell specific knockout of specific NF-κB subunits will be required to address this question. The target cell type that responds to TSLP and suppresses IL-22 production by ILCs also remains to be identified. Dendritic cells (DCs) are a likely candidate, as TSLP affects DC polarization and cytokine production, which could in turn affect IL-22 expression by ILCs. Moreover, TSLP neutralization only partly restored immunity to *C. rodentium* in IKKα$^{\Delta IEC}$ mice, suggesting that additional, as yet unknown, mechanisms also contribute. From a clinical perspective, this study raises the interesting possibility that TSLP neutralization might have beneficial effects in enteropathogenic bacterial infections, although the diverse biological functions of TSLP may make it difficult to exploit this newly identified mechanism therapeutically.



Upon infection with *C. rodentium*, IKKα–deficient IECs increase their production of TSLP, which inhibits IL-22 production by RORγt^{+} ILCs indirectly by acting on an as yet unknown cell type (possibly dendritic cells). Diminished IL-22 production in turn decreases expression of the antimicrobial proteins REG3β and REG3γ and impairs epithelial regeneration.

Giacomin, P.R., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141831

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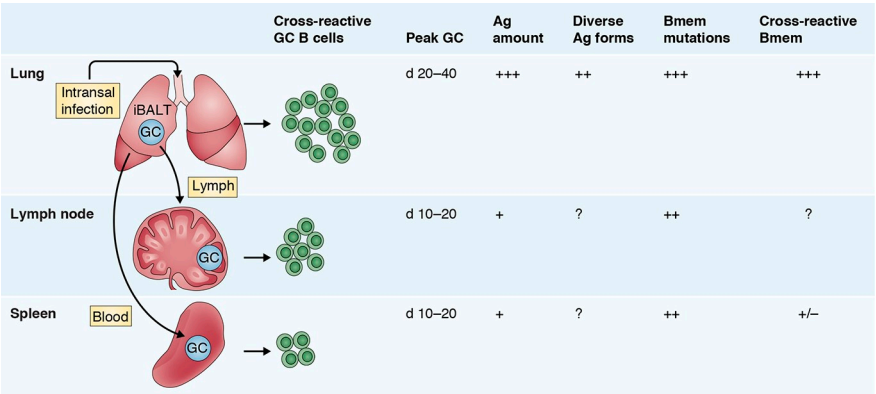
Getting close to the action elicits better memories

Insight from Jason Cyster

Induction of broadly neutralizing antibodies against rapidly evolving viruses is a "holy grail" of current vaccine research. A study in this issue reveals that it's not just the properties of the immunogen that matter, but also the anatomical site of the B cell response.

Influenza infection causes substantial morbidity and mortality each year despite the wide use of an annually updated vaccine. A major limitation of the current vaccination approach is the weak ability to induce antibodies that recognize variant forms of the hemagglutinin (HA) antigen, which arise continually by antigenic drift (point mutations in an existing strain) and more rarely by antigenic shift (major changes in the HA gene by viral mixing). In this issue, Adachi et al. examine the requirements for the generation of cross-reactive antibodies that recognize different influenza strains. By comparing the properties of germinal center (GC) and memory B cells (Bmem) isolated from lung, draining LNs, and spleen of intranasally infected mice, they discovered that cross-reactive B cells are preferentially generated in the lung. Using conditional Bcl6 ablation, they established that ongoing GC responses are needed for generation of these late-arising cross-reactive memory cells. Using a novel procedure of intratracheal exposure to low amounts of 5-ethynyl-20-deoxyuridine (EdU) to locally label dividing cells, they show that cross-reactive Bmem are preferentially generated and maintained in the lung.

Although GCs are most readily induced in secondary lymphoid organs, they can also form ectopically at sites of inflammation. Remarkably, this study shows that ectopic GC formation doesn't just provide additional antibody-generating capacity, it provides a qualitatively unique environment tailored for the induction of cross-reactive antibodies. As the authors suggest, the enhanced cross-reactive Bmem induction in the lung might reflect the presence of greater amounts of viral antigen, drifted variants that arose before the virus was cleared, or forms of HA that are more accessible to the BCR. It will be exciting to see whether these factors operate together to make the local response the most efficacious.



Anatomical differences that may contribute to induction of influenza strain cross-reactive B cells in the lung. The diagram on left shows paths of antigen (Ag) travel and sites of GC formation (iBALT, induced bronchial-associated lymphoid tissue, appears about 1 week after infection), which differ in the generation of HA-binding, cross-reactive GC B cells (green) at day 30 after intranasal infection with influenza A virus X31. The table summarizes key differences between sites, including the timing of the peak GC response, amount and form of Ag remaining at day 30, Ig mutation load (Bmem mutations), and the presence of cross-reactive Bmem. Data on the peak GC response, Ig mutation load, and cross-reactive B cell frequencies are based on the findings reported in Adachi et al.

Interestingly, the proliferation rate of HA-binding GC B cells in the lung was greater than in LNs and spleen. The basis for this effect was not examined but might reflect properties associated with the inflammatory milieu or perhaps even the higher oxygen levels in the lung. Whatever the explanation, the faster proliferation rate may contribute to more rapid accrual of mutations and possibly greater diversification of the Bmem pool that is generated. While the mechanistic studies move forward, this work is a reminder that anatomy and not just molecular components matter in the efforts to develop vaccine strategies that induce broadly neutralizing antibody responses.

Adachi,Y., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20142284

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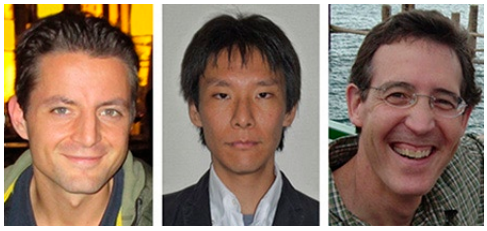
Leukotrienes and the other airway: Celiac disease and asthma collide



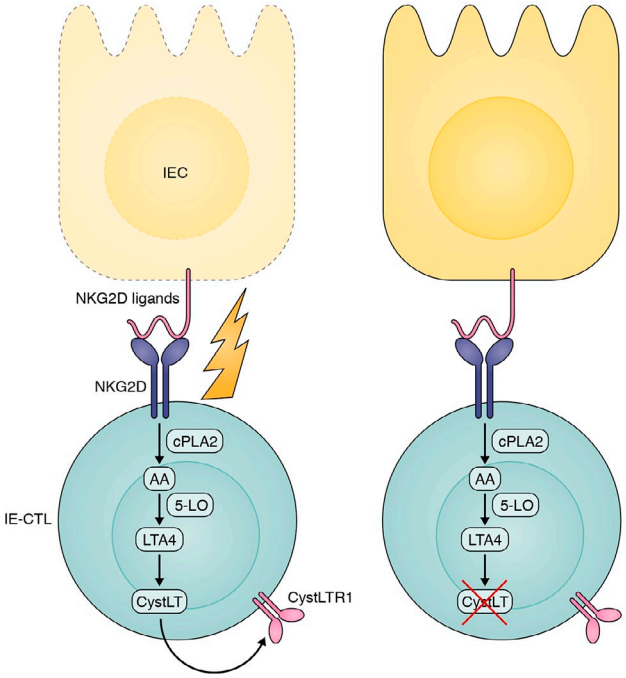
Insight from (left to right) Joep Grootjans, Shuhei Hosomi, and Richard Blumberg

Celiac disease (CD) arises from inflammatory T cell and antibody responses to dietary gluten in a genetically susceptible host. CD is notable for a distinct pattern of histological injury to intestinal epithelial cells (IECs) characterized by crypt hyperplasia and atrophy of the villi, which is caused by infiltrating intraepithelial cytotoxic $CD8^+$ T cells (IE-CTLs).

CD is antigen driven and strongly restricted to particular MHC class II alleles such that antigen (gluten) elimination is a recognized therapy. However, emerging data suggest that the "final hit" to disease progression is through downstream noncognate events that are orchestrated by the MHC class II response and may be part of a common spectrum of immune pathways shared with multiple diseases such as inflammatory bowel disease, graft-versus-host disease, and infectious enteritis. Specifically, gluten promotes interleukin-15 (IL-15) expression by distressed IECs via yet to be defined mechanisms. IL-15 in turn recruits additional IE-CTLs to the epithelium and arms these cells by increasing the expression of the activating receptor natural killer group 2 member D (NKG2D). NKG2D ligands, such as MHC class I chain related A and B (MICA/B), are displayed on stressed cells, resulting in their cytolysis by the NKG2D-expressing IE-CTLs, thus setting up a continuous cycle of IEC destruction.

In this issue, Tang et al. follow up on previous observations showing that NKG2D-mediated cytolysis depends on intracellular signaling via cytosolic phospholipase A2 (cPLA2), which catalyzes release of arachidonic acid (AA) from the membrane. Unexpectedly, they find that the NKG2D-induced cPLA2 and IL-15 signaling pathways converge on the production of leukotrienes, AA metabolites that have been previously linked to allergic disorders including asthma. They find that NKG2D ligation up-regulates an enzymatic cascade associated with 5-lipoxygenase and leukotriene C4 synthase (LTC4S), leading to production of cysteinyl leukotrienes (CystLTs) and the expression of the CystLT receptor on effector cells. Not only do they demonstrate that pharmacological inhibition or siRNA knockdown of enzymes involved in the CystLT pathway significantly impair NKG2D-mediated killing, they show that CystLT up-regulation is demonstrable in patients with active CD but not in healthy controls or in patients on a gluten-free diet.

An FDA-approved CystLT inhibitor, montelukast, is beneficial in patients with asthmatic disease, and the authors show that in vitro pretreatment of IE-CTL lines with montelukast significantly decreases their cytolytic capacity. These experiments unravel an unexpected role for CystLTs in NKG2D-mediated cytolysis and also open up a potential new therapeutic approach for CD that is eminently testable. These exciting results not only underline the benefits of careful and painstaking interrogation of complex signaling pathways but also the opportunities that can be derived from applying clinical insights across widely disparate organ-associated diseases.



Interaction of NKG2D on IE-CTLs with its ligands expressed on distressed IECs results in a cascade of reactions ultimately leading to increased levels of CystLTs, which is responsible for IEC killing, as observed in patients with CD. Inhibiting CystLTs might decrease IE-CTL cytotoxic capacity and could therefore be an interesting new therapeutic approach in patients with CD.

Tang, F., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150303

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