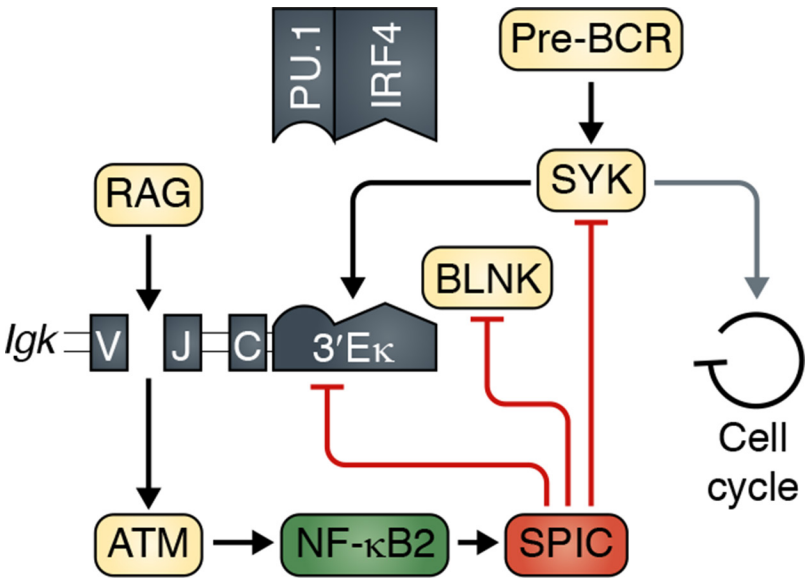
Pumping the breaks on B cell development

Insight from Stephen Desiderio

The assembly of immunoglobulin genes occurs in ordered waves during B cell development. The heavy chain (*Igh*) locus generally recombines first, and each allele has at most one chance to undergo a productive rearrangement. Subsequently, at the κ (*Igk*) locus, individual alleles can undergo sequential rounds of rearrangement, permitting different light-heavy chain combinations to be tested until a functional, nonself-reactive immunoglobulin is produced. The two waves of recombination are separated by a checkpoint governed by the pre–B cell receptor (pre–BCR), which enforces allelic exclusion at the *Igh* locus, triggers proliferation, and promotes *Igk* rearrangement. This raises the question: how do we mitigate the genomic damage that might occur if DNA cleavage and cell cycle entry were initiated simultaneously? In this issue, Bednarski et al. suggest a solution: an unexpected mechanism by which RAG-induced DNA double-strand breaks (DSBs) suppress pre–BCR signaling.

The authors began by identifying genes that undergo RAG-dependent changes in expression as progenitors progress from the large pre–B stage to the small pre–B stage, at which *Igk* rearrangement begins. Of particular interest were the genes encoding RELB and p100, components of the transcription factor NF-κB2. Because RELB and p100 are induced by DSBs through the activation of ataxia-telangiectasia mutated (ATM), they seemed well positioned to act as a bridge between RAG and downstream transcriptional targets.

Indeed, many of the genes regulated by RAG-dependent DSBs were targets of NF-κB2. One of these targets encodes the transcriptional repressor SPIC, which had been implicated as a negative regulator of B cell development. Bednarski et al. were able to situate RAG activity at the apex of a signaling cascade that induces SPIC through sequential activation of ATM and NF-κB2. SPIC was found to close the circle, inhibiting expression of the pre–BCR signal mediators SYK and BLNK by antagonizing PU.1 at their corresponding genes. The resulting extinction of pre–BCR signaling enforces cell cycle arrest in G1 while suppressing κ rearrangement.



RAG-induced DSBs trigger a signalling pathway that culminates in the suppression of pre–BCR signals by the transcriptional repressor SPIC.

The ability of RAG-induced DSBs to suppress pre–BCR signaling may explain how κ rearrangement is punctuated in pre–B cells: (1) an initial round of κ rearrangement in small pre–B cells generates DSBs; (2) DSBs, acting through ATM, induce SPIC and turn down pre–BCR signaling, inhibiting κ rearrangement and enforcing G1 arrest; and (3) repair of the first wave of DSBs extinguishes ATM signaling and restores pre–BCR signaling, initiating another round of κ rearrangement. The model, which is attractive in its economy, may inform development of therapies that interrupt pre–BCR signaling in malignancies of pre–B cell origin, particularly acute lymphoblastic leukemias.

Would interruption of the linkage between RAG DSBs and pre–BCR signaling promote genomic instability? Other mechanisms, notably activation of p53 and destruction of RAG-2 at the G1-to-S transition, are in place to segregate DSBs from S phase, but these may not be sufficiently genoprotective in the face of persistent pre–BCR signaling. In this regard, it will be interesting to assess genomic instability in SPIC-null mice, particularly in a p53-deficicient setting.

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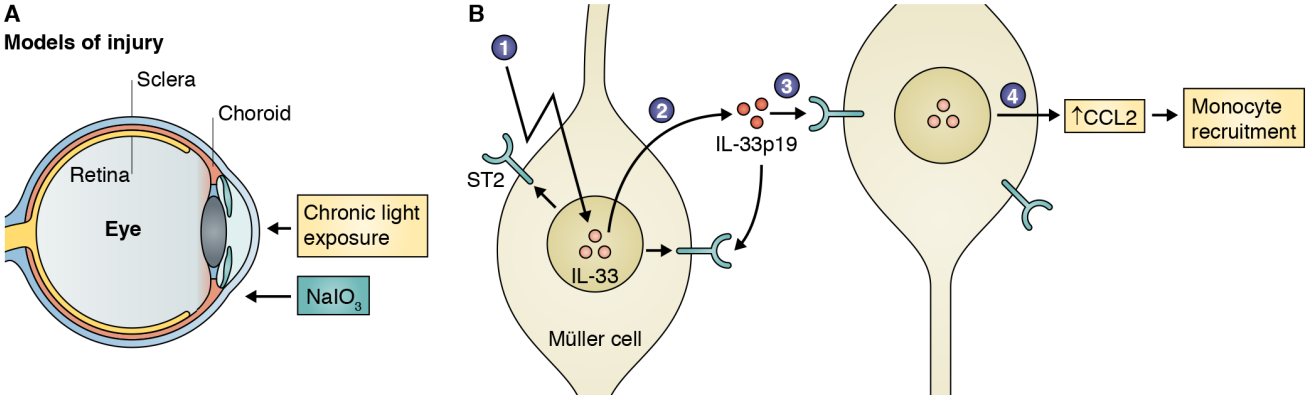
Shedding light on IL-33 in the eye

Insight from Sachin Gadani (left) and Jonathan Kipnis (right)

In this issue of *JEM*, Xi et al. describe how inflammation is initiated in the retina, studying the alarmin IL-33. Expressed in healthy tissues and released in conditions of cell damage or stress, alarmins include molecules such as ATP, HMGB1, IL-1α, and IL-33. IL-33 is a nuclear protein highly expressed in skin and lung epithelial cells, CNS oligodendrocytes, and elsewhere, and is typically released by necrotic cell death to "sound the alarm" for immune cells. Interestingly, its expression in the CNS is among the highest of any tissues, although its role there has only recently begun to be studied. IL-33 is known to influence outcome of EAE, has effects on pain perception, and we and others have demonstrated its importance after traumatic CNS injury.

The current work sheds light on IL-33 in the eye, addressing the cellular localization and function of released IL-33 after retinal injury or stress. The authors show that after stressful stimuli, Müller cells release IL-33, which activates them in an autocrine manner and leads to CCL2 expression and subsequent macrophage recruitment.

IL-33 is classically thought to be released by necrotic cell death, in part because its lack of a secretory signal peptide, but numerous recent studies are challenging this notion. Contributing to this evidence, the authors demonstrate that Müller cells stressed in vitro release IL-33 without death. IL-33 is released by Müller cells as a 19-kD cleavage product, detectable by Western blot in both the nuclear and cytoplasmic fractions after cell stress.



After retinal injury or stress, Müller cells produce the alarmin IL-33, leading to autocrine stimulation and monocyte recruitment through CCL2 secretion.

Though expressed widely, the highest producers of IL-33 are in the skin, lung, and CNS. Skin and lung—barrier tissues, with the frequent threat of traumatic injury—must express alarmins to help rapidly mobilize a response. But why is IL-33 expressed so highly in the CNS? IL-33 presumably acts as an activator of CNS inflammation, but from an evolutionary perspective, it is hard to believe that any trait would develop and remain only for benefit during overt CNS trauma. Perhaps the real role for IL-33 is in more subtle and common CNS insults. For example, the retina is frequently exposed to light, which could cause frequent but minor cellular damage. Given the ability for Müller cells to secrete IL-33 without dying, the role of IL-33 in the healthy eye may be to balance the minor cell damage induced upon exposure to high intensity light. Alternatively, IL-33 in the CNS could have a novel function to that in the periphery. The CNS frequently reuses "immune" molecules for other purposes, such as MHC1 in synaptic plasticity or complement in synaptic pruning. Previous discoveries of context-dependent functions for "immune" molecules in the brain teach us to expect the unexpected, and we must remain open minded when studying IL-33, or any other immune molecule, in the CNS.

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