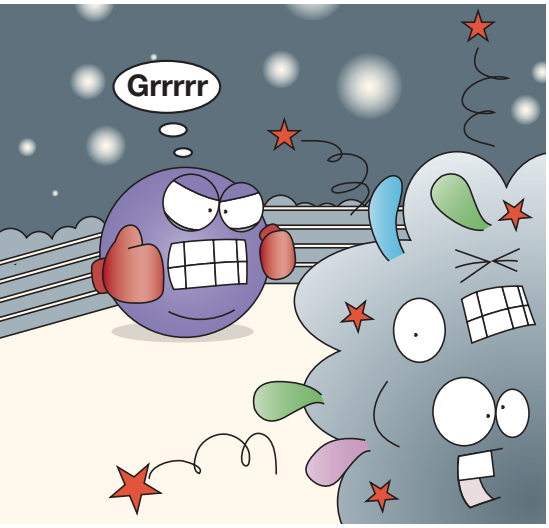
HIV

APOBEC3F joins the fight



Two new papers published in *Current Biology* show that human APOBEC3F joins APOBEC3G as a second retroviral-restriction factor potentially able to inhibit HIV-1 infectivity *in vivo*. APOBEC3G was identified in 2003 as causing deamination of cytosine (C) to uracil (U) in minus-strand viral cDNAs and therefore causing a switch from guanine (G) to adenine (A) on the plus strand. The accumulation of a high level of G to A mutations significantly inhibits viral infectivity, so APOBEC3G seems to be the basis of part of our innate immune defence against retroviruses.

However, when HIV-1 samples from patients with AIDS are sequenced, it is clear that APOBEC3G cannot account for all of the G to A mutations that are observed. APOBEC3G has a strong preference for minus-strand CC as a substrate (where the mutated nucleotide is underlined), equating to GG on the plus strand, but both GG and GA are heavily favoured for substitution in patient samples. Humans encode eight other putative members of the cytosine-deaminase family, and given the marked homology between APOBEC3F and APOBEC3G, it was proposed that APOBEC3F could function similarly to APOBEC3G and account for the GA mutations.

Michael Malim and colleagues sequenced HIV cDNAs from infected cells that were cultured in the presence or absence of APOBEC3G and APOBEC3F. Having shown that APOBEC3F does induce G to A mutations that correlate with loss of infectivity, they looked at the local consensus sites for cytosine deamination. The preferred target for APOBEC3F was minus-strand TC, which correlates with the HIV GA mutations seen in patients. Reuben Harris and colleagues independently showed that 70% of the APOBEC3F-preferred dinucleotides were TC, whereas 87% of the APOBEC3G-preferred dinucleotides were CC. Both groups showed that the expression pattern of APOBEC3F includes human T cells, which account for most of the host cells infected *in vivo*. So, APOBEC3G and APOBEC3F both seem to be capable of functioning in innate defence against HIV infection but have clearly distinct dinucleotide preferences. Harris and colleagues further showed that the infectivity decreases attributable to APOBEC3F and APOBEC3G were additive, indicating that they can function independently.

However, the two studies differed in terms of the extent to which APOBEC3F is sensitive

T-CELL DEVELOPMENT

Mi-2β acting positively

New research published in *Immunity* indicates that the ATP-dependent chromatin remodeller Mi-2β — which was previously thought to be a negative regulator of gene expression — is a positive regulator of CD4 expression by double-positive (DP) thymocytes and is required for thymocyte transition from the late double-negative (DN) stage to the DP stage of development.

The nucleosome-remodelling histone deacetylase (NuRD) complex associates with Ikaros proteins, which are known regulators of T-cell development. The complex comprises several proteins, including Mi-2. There are two homologues of Mi-2, of which Mi-2β is the most highly expressed in thymocytes. So, to study the role of the Mi-2-containing chromatin-remodelling complex in T-cell development, Williams *et al.* generated mice in which Mi-2β was specifically inactivated at the DN stage of thymocyte development and remained inactive in mature T cells.

In the Mi-2β-deficient mice, thymic cellularity was reduced, as was the absolute number of DP thymocytes. By contrast, the number of DN thymocytes was normal, and further analysis revealed an accumulation of DN4 thymocytes (that is, those cells in the final stages of DN development), indicating that Mi-2β regulates the DN4 to DP transition during thymocyte differentiation.

In addition to the decreased numbers of DP thymocytes in the thymi of the Mi-2β-deficient mice, the percentage of these cells in each thymus was also markedly reduced. By contrast, the proportion of $CD8^+$ cells was increased. However, the phenotype of these $CD8^+$ T cells resembled that of wild-type DP thymocytes, leading the authors to suggest that the $CD8^+$ cells that accumulate in the absence of Mi-2β are DP cells that fail to express CD4 appropriately. Consistent with the hypothesis that Mi-2β regulates *Cd4* gene expression, in mice transgenic for a reporter driven by the *Cd4* enhancer and promoter, expression levels of the reporter in thymocytes were reduced in the absence of Mi-2β, with the lowest levels of expression being detected in $CD8^+$ cells — the cells in which CD4 was not expressed appropriately. Furthermore, in wild-type thymocytes, Mi-2β was shown to associate directly with the *Cd4* proximal enhancer — a regulatory element known to be required for *Cd4* gene expression during thymocyte development.

to inhibition by the viral protein Vif. Vif protects HIV from cytosine deamination by APOBEC3G by targeting it for proteasomal degradation. So, HIV produced in the presence of APOBEC3G and Vif retains infectivity. Malim and colleagues showed that in the presence of Vif and APOBEC3F, more than 90% of the HIV sequences had zero or only one mutation, whereas when Vif was removed from the system, this dropped to less than 50%. Therefore, APOBEC3F is susceptible to inhibition by Vif. By contrast, Harris and colleagues showed that Vif could only partially restore the infectivity of viruses that were produced in the presence of APOBEC3F, indicating that APOBEC3F is less susceptible to Vif than APOBEC3G. Further study is required to discover whether and how APOBEC3F might avoid Vif-mediated proteasomal degradation.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPERS Bishop, K. N. *et al*. Cytidine deamination of retroviral DNA by diverse APOBEC proteins. *Curr. Biol.* 24 June 2004 (doi:10.1016/S0960982204004683). | Liddament, M. T., Brown, W. L., Schumacher, A. J. & Harris, R. S. APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 *in vivo*. *Curr. Biol.* 24 June 2004 (doi:10.1016/S096098220400466X).

The *Cd4* proximal enhancer normally shows high levels of histone H3-acetylation, but this was reduced in the thymi of Mi-2β-deficient mice, probably as a result of the observed decrease in association of the histone acetyltransferase p300 with the enhancer. Subsequent analysis showed that p300 and Mi-2β associate with the E-box-binding protein HEB. Because the *Cd4* enhancer contains E-box binding sites, the authors propose that Mi-2β is recruited to the enhancer through its interactions with HEB. Through its ATP-dependent chromatin-modelling function, Mi-2β stabilizes HEB binding, such that associated/recruited p300 increases local histone acetylation, locking the chromatin in an open conformation and thereby leading to *Cd4* gene expression.

This study identifies Mi-2β as crucial for thymocyte differentiation at the transition from the DN4 to the DP stage of development and for regulation of *Cd4* gene expression in DP thymocytes. As Mi-2β has previously been defined as a negative regulator of gene expression, this study uncovers a new role for Mi-2β, and future studies will probably characterize more situations in which it can act positively.

Karen Honey

References and links

ORIGINAL RESEARCH PAPER Williams C. J. *et al*. The chromatin remodeler Mi-2β is required for CD4 expression and T cell development. *Immunity* **20**, 719–733 (2004).

T-CELL SIGNALLING

Getting faster with experience

Like a racing driver navigating a familiar course, T cells respond faster to a second exposure to their specific antigen. But what makes an experienced T cell go faster? This study in *The Journal of Immunology* indicates that crucial differences between naive and memory T cells in terms of the formation and duration of immunological synapses might be responsible.

The immunological synapse is the region of contact between a T cell and an antigen-presenting cell (APC), consisting of a central supramolecular activation cluster (cSMAC) and a peripheral SMAC (pSMAC). Previous experiments have shown that when a naive T cell first encounters an APC presenting the antigen for which it is specific, the immature synapse that first forms contains adhesion molecules in the cSMAC and T-cell receptor (TCR)-signalling molecules in the pSMAC. This pattern then reverses as the mature synapse forms so that signal transduction occurs in the cSMAC.

Watson and Lee wanted to see if the same holds true for memory T cells. They isolated naive and memory $CD4^+$ T cells from DO11.10 mice, which express a transgenic TCR specific for an ovalbumin (OVA) peptide. These were then incubated with OVA-pulsed APCs. For naive T cells, as expected, TCRs and the signalling molecule protein kinase C-θ were found in the pSMAC 5 to 15 minutes after contact with an APC, whereas the adhesion molecule lymphocyte function-associated antigen 1 (LFA1) was present in the cSMAC. This pattern had reversed by 30 minutes to form the mature synapse, but by 60 to 90 minutes, the whole synapse had dissipated. However, for memory T cells, the mature synapse had formed by 5 to 15 minutes after initial APC contact and remained for more than 90 minutes. In the memory T cells only, TCRs were constitutively associated with lipid rafts — regions of the membrane that accumulate in the synapse — which might account for the more rapid formation of the mature synapse.

Next, they looked at the effects on TCR signalling. In naive T cells, it is known that LCK is active in the pSMAC of the immature synapse but not when the mature synapse has formed. By contrast, they showed that in memory T cells, LCK was still active when it moved into the cSMAC of the mature synapse. This might be due to the observed presence of the tyrosine phosphatase CD45 — which promotes dephosphorylation of an inhibitory tyrosine of LCK — in the cSMAC of memory but not naive T cells. CD45 was also constitutively associated with TCRs in lipid rafts in memory T cells only.

However, although Lck activity was prolonged in memory T cells, this did not affect the downstream kinase ZAP70 (ζ-chain-associated protein kinase of 70 kDa). The authors therefore suggest that prolonged LCK activity might induce alternative memory-cell-specific signalling. The downstream effects of these differences in synapse formation and composition clearly require further investigation.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER Watson, A. R. O. & Lee, W. T. Differences in signaling molecule organization between naive and memory $CD4^+$ T lymphocytes. *J. Immunol.* **173**, 33–41 (2004).