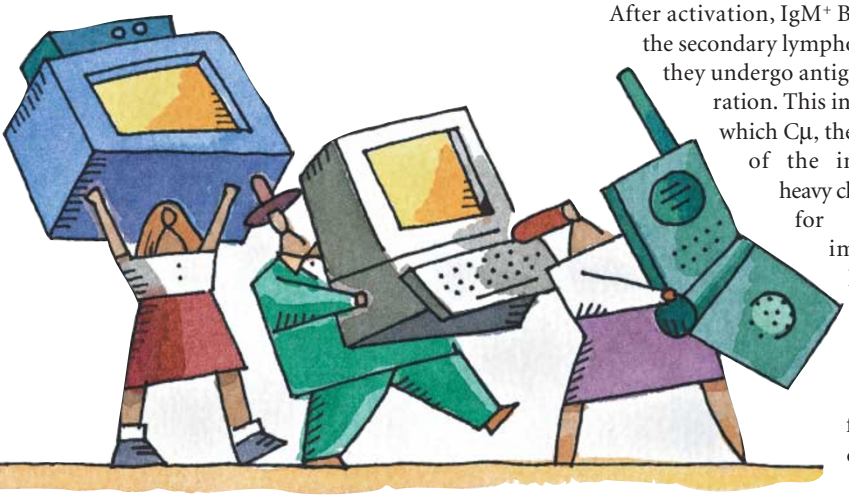
B CELLS

Antibody responses held up



B-cell exposure to antigen drives diversification of the antibody repertoire through class-switch recombination (CSR) and somatic hypermutation (SHM). Although the molecular mechanisms of CSR and SHM are the focus of many studies, the transcription factors that programme B cells to undergo these processes are less well characterized. New light has now been shed on this by a recent study published in *Nature* that identifies the B-cell-specific transcription repressor BACH2 as crucial for CSR and SHM.

After activation, IgM^+ B cells migrate to the secondary lymphoid organs where they undergo antigen-driven maturation. This involves CSR — in which Cμ, the constant region of the immunoglobulin heavy chain, is exchanged for an alternative immunoglobulin heavy-chain constant region such that B cells produce antibodies of the same specificity but with distinct effector functions — and SHM, in which point mutations introduced into the variable region of immunoglobulin heavy and light chains enable the selection of B cells that generate antibodies of higher affinity for antigen.

Immunohistochemical analysis of transcription-factor expression revealed that BACH2 is expressed by IgM^+ cells within the lymphoid follicles of the spleen — the site of CSR and SHM. By contrast, no BACH2 was detected in IgM^+ marginal-zone B cells. To determine whether this pattern of expression reflected a role for BACH2 in the antibody response, Muto *et al.* generated BACH2-deficient mice. Normal numbers of $B220^+IgM^+IgD^-$ naive B cells were observed in the spleens of *Bach2*$^{-/-}$ animals; however, these mice had markedly fewer $B220^{hi}IgM^{low}IgD^+$ mature B cells. Consistent with this, when compared with control animals, BACH2-deficient mice had higher levels of IgM and lower levels of other immunoglobulin isotypes (such as IgG1 and IgG2a) in the serum. Furthermore, BACH2-deficient mice failed to mount antigen-specific IgG3 or IgG1 antibody responses after immunization with a T-cell-independent antigen or a T-cell-dependent antigen, respectively. By contrast, antigen-specific IgM production and differentiation of IgM plasma cells was normal, implicating BACH2 as a regulator of CSR. A role

ANTIGEN PRESENTATION

Preferential treatment

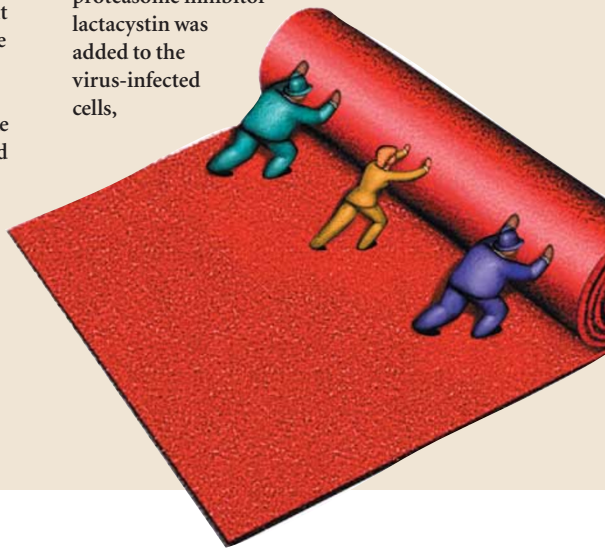
Two new papers in *Science* this month have shed light on the mechanism of cross-priming, by indicating the types of antigen that are favoured by this process. Both groups suggest that this has important implications for vaccine design.

Peptides presented on MHC class I molecules to $CD8^+$ cytotoxic T lymphocytes (CTLs) are normally derived from endogenous proteins, such as viral proteins produced by infected host cells. In some cases, exogenous proteins can also be taken up by MHC class-I-positive cells for cross-presentation. This might be particularly important for naive CTL priming, which requires professional antigen-presenting cells (pAPCs) and cannot be achieved by other tissue cells.

Ton Schumacher and colleagues looked at whether the location of a CTL epitope within a peptide affects cross-presentation. Signal peptides — the amino-terminal part of a protein that targets it to the endoplasmic reticulum and is then cleaved — are an important endogenous source of MHC class-I-restricted antigens. But, in their system, an epitope present in the functional signal sequence of a GFP fusion protein could not be cross-presented to CTLs at any significant level, whereas the same epitope or a different epitope close to the carboxyl terminus of the mature GFP protein could be efficiently cross-presented. They showed that this difference was due to the efficiency of uptake by pAPCs. When naive mice were challenged with cells transfected with a GFP construct containing two different epitopes (one in the signal sequence and one in the mature protein), T-cell priming *in vivo* was skewed towards the epitope in the mature protein. This shows that the exogenous pathway is dominant for the priming of naive CTLs in this system.

The authors suggest that cross-priming is biased towards unprocessed antigens and against sequences that are degraded rapidly after synthesis, such as signal peptides. This ties in nicely with the second study, by Jonathan Yewdell and colleagues, which shows that cross-priming favours proteasome substrates rather than pre-processed peptides.

MHC class-I-negative cells, which cannot present endogenous antigen, were infected with recombinant vaccinia virus encoding full-length ovalbumin (OVA) and then introduced into mice that had received ovalbumin-specific $CD8^+$ T cells. When the proteasome inhibitor lactacystin was added to the virus-infected cells,



for BACH2 in SHM was also indicated by the observation that the frequency of mutations in the immunoglobulin regions that are crucial for antigen binding was decreased in *Bach2*$^{-/-}$ mice immunized with a T-cell-dependent antigen.

CSR requires germline transcription through the immunoglobulin heavy-chain regions that are being exchanged, followed by excision of the DNA between the two regions. By analysing the products of these two processes, it is possible to examine the steps of CSR. BACH2-deficient B cells stimulated to undergo CSR *in vitro* were able to generate the germline transcripts indicative of the transcription step of CSR. However, detection of the excised DNA was markedly decreased, indicating that BACH2 deficiency prevents cleavage and/or ligation of the switch regions.

This report defines BACH2 as a new regulator of CSR and SHM, and undoubtedly, further research will centre on the identification of the B-cell genes that are controlled by BACH2, as well as the mechanisms by which B-cell activation regulates BACH2 activity.

Karen Honey

References and links

ORIGINAL RESEARCH PAPER Muto, A. *et al.* The transcriptional programme of antibody class switching involves the repressor Bach2. *Nature* **429**, 566–571 (2004).

activation of the CD8$^+$ T cells was not prevented, indicating that cross-priming can occur in the absence of proteasomal degradation of the protein in the donor cells. By contrast, when donor cells were infected with recombinant vaccinia virus encoding a particular OVA epitope, CTLs specific for the OVA epitope could not be detected, showing that minimal peptides cannot be cross-presented. Similarly, a chimeric protein designed to be rapidly degraded by the proteasome could not be cross-presented unless the cells were first treated with lactacystin to prevent degradation. The authors therefore suggest that in their *in vivo* set-up, cross-priming is based on the transfer of proteins rather than peptides, which casts doubt on the previously suggested role of peptide-binding molecular chaperones in this process. Together, the two studies show that for efficient cross-presentation, vaccine antigens should be engineered for maximum half-life.

Kirsty Minton

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INSECT IMMUNITY

Distinct recognition

Although it is clear that the response of *Drosophila* to Gram-negative bacteria is mediated by the Immune-deficiency (IMD) pathway, the identity of the microbial components that initiate signalling remains controversial. One previous report indicated that polymeric peptidoglycan was the immunostimulatory component and that lipopolysaccharide (LPS) and monomeric peptidoglycan had no such activity, whereas a second study concluded that both LPS and peptidoglycan could activate the IMD pathway. Now, in a recent paper published in *Immunity*, Neal Silverman and colleagues have shown that LPS cannot stimulate the IMD pathway, whereas polymeric and monomeric Gram-negative peptidoglycan can.

Kaneko *et al.* initially found that both re-extracted *Escherichia coli* LPS and Gram-negative peptidoglycan were potent inducers of the IMD pathway in cultured cells. However, treatment of the LPS preparation with the peptidoglycan-degrading enzymes mutanolysin or PGRP-SC1B reduced its stimulatory capacity by 10- or 100-fold, respectively, and the IMD-stimulatory activity could be separated from LPS by fractionation of the mutanolysin-digested LPS preparation. These results indicate that LPS does not activate the IMD pathway in *Drosophila* cells and that stimulation by the original re-extracted LPS preparation was a result of peptidoglycan contamination.

The observation that digested peptidoglycan contaminants in the LPS preparation triggered the IMD pathway indicated that small fragments of peptidoglycan could be immunostimulatory. Consistent with this, a monomeric disaccharide–tetrapeptide fragment of peptidoglcyan was shown to activate *Drosophila* cells and to induce antimicrobial-gene expression in adult flies. Similarly, synthetic Gram-negative peptidoglycan-like lactyl-tetrapeptides triggered IMD-pathway activation in *Drosophila* cells. By contrast, lactyl-tetrapeptides that resembled Gram-positive peptidoglycan (because they contained a lysine residue instead of a diaminopimelic-acid residue at the third position of the stem peptide) were not immunostimulatory, indicating that *Drosophila* cells distinguish between monomeric peptidoglycan structures.

Peptidoglycan-recognition protein LC (PGRP-LC) is a receptor known to be required for antimicrobial Gram-negative immune responses in *Drosophila*. Expression of the three distinct isoforms of this receptor, PGRP-LCa, -LCx and -LCy — which have unique extracellular domains — was reduced using isoform-specific RNA interference. Cells in which the expression of PGPR-LCx was reduced were markedly impaired in their ability to activate antimicrobial-gene expression when exposed to polymeric Gram-negative peptidoglycan, whereas knockdown of PGRP-LCa expression had no effect on this response. By contrast, recognition of the monomeric disaccharide–tetrapeptide fragment of peptidoglcyan required PGRP-LCa and PGRP-LCx.

This report shows that LPS cannot stimulate the *Drosophila* IMD pathway and that the immunostimulatory effect of LPS preparations is a result of contaminating peptidoglycan. In addition, both polymeric and monomeric Gram-negative peptidoglycan can activate the IMD pathway, but they are recognized by distinct isoforms of the PGRP-LC receptor. As mammals have four PGRP homologues, the authors suggest that this study might provide insight into immune recognition by these receptors.

Karen Honey

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