IMMUNOGENETICS

Linking genetics and resistance to infection

All immunologists know the old adage that MHC polymorphisms are believed to benefit individuals by allowing the presentation of a broad array of antigenic peptides to T cells. But, direct evidence to support this proposed link between MHC haplotype and resistance to infection has been lacking so far. Now, Janko Nikolich-Žugich's lab provides evidence that, in contrast to this widely held view, MHC polymorphisms can influence immune defence by allowing the best T-cell precursors to be selected from a diverse T-cell receptor (TCR) repertoire.

To investigate how MHC polymorphisms influence MHC class-I-restricted T-cell responses, the authors used the mouse strains C57BL/6 (B6; MHC haplotype H-2^{b}) and B6.C-H-2bm8 (bm8; MHC haplotype H-2^{bm8}), the MHC molecules of which differ by only four amino acids located in the floor of the peptide-binding groove. MHC class I molecules from both strains bind HSV-8p, an immunodominant peptide from glycoprotein B of *Herpesvirus hominis* type 1 (HVH1).

When challenged with HVH1, bm8 mice were 3–4 times more resistant to infection than were B6 mice. When $CD8^{+}$ T cells were depleted from the mice, no differences between the strains were observed in terms of resistance to infection, which indicates that MHC class-I-dependent immune responses govern resistance to infection. In (B6 × bm8) F_2 hybrid mice, a single copy of H-$2K^{bm8}$ conferred enhanced resistance.

In both B6 and bm8 mice, ~90% of cytotoxic T lymphocyte (CTL) responses to HVH1 are specific for the immunodominant peptide HSV-8p. Transfer of HSV-8p-specific T cells from bm8, but not B6, mice into F_1 hybrids conferred significant protection against infection, which indicates that differences in a single allele can enhance protection in a T-cell-dependent manner.

How does this monoallelic difference lead to enhanced protection? Differential peptide binding to MHC molecules was not the answer, because the kinetics of binding of HSV-8p to H-$2K^{b}$ and to H-$2K^{bm8}$ were identical. The possibility that H-$2K^{b}$-mediated negative selection interferes with resistance was ruled out also.

Because H-$2K^{bm8}$ positively selects $CD8^{+}$ T cells with a broader TCR repertoire than does H-$2K^{b}$, the authors next decided to investigate the influence of the TCR repertoire on the quantity and quality of HSV-8p-specific CTL responses. No quantitative differences in the numbers of CTL precursors that were mobilized in response to infection were observed between the strains. In B6 mice, the CTL repertoire is dominated by TCRs with two variable-region (V) β-chains, Vβ8 and Vβ10, whereas bm8 mice use these Vβ chains plus five others. The number of $V\beta8^{+}$ T cells is similar in each strain. If the magnitude of the CTL response is an important parameter, depletion of $V\beta8^{+}$ T cells — which comprise ~15–25% of the total response to

INNATE IMMUNITY

Zebrafish give a clearer view

Granulomas are organized structures that typically form during mycobacterial infections as a result of complex interactions between mycobacteria and the host. By the time that they are first detectable in mammals, granulomas contain both macrophages and T cells — so a longstanding puzzle has been whether granuloma formation is initiated by innate or adaptive immunity. Now, greater transparency (literally) has been provided by the zebrafish.

The embryos and early swimming larvae of zebrafish are optically transparent, making them an ideal model in which to observe pathogen–host interactions in real time. Adult zebrafish, which have both macrophages and T cells, can form granulomas in response to infection with *Mycobacterium marinum*, which is a close relative of the human pathogen *Mycobacterium tuberculosis*. But, do granulomas form in zebrafish embryos, at which stage only macrophages are present?

Muse Davis and co-workers injected zebrafish embryos intravenously with fluorescently labelled *M. marinum* then observed them by video microscopy. Three days later, infected macrophages could be seen to have extravasated into the tissues, and they had begun to aggregate — squeezing together to form clusters with the typical morphology of granulomas. Heat-killed *M. marinum* and *Salmonella arizonae* failed to induce the formation of such granuloma-like structures, which indicates that this process is triggered specifically by mycobacteria–host interactions.

To confirm that these were *bona fide* granulomas, the authors looked at the expression of *M. marinum* genes that had been characterized previously as being activated in granulomas (granuloma-activated genes, GAGs) or after infection of cultured macrophages (macrophage-activated genes, MAGs). All four MAGs of *M. marinum* were activated soon after phagocytosis by macrophages, but the three GAGs were activated only once the infected macrophages had aggregated. Therefore, the complex bacteria–host interactions that occur in adult granulomas are mimicked in the embryo.

So, it seems that innate immune factors are sufficient to initiate granuloma formation — which goes against previous studies that cast T cells in the leading role. This is the first time that pathogen–host interactions have been observed *in vivo*, as they happen. And this study establishes zebrafish embryos as an illuminating model in which to study granuloma formation.

Jennifer Bell

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WEB SITE

Lalita Ramakrishnan's lab:
http://depts.washington.edu/daid/faculty/ramakrishnan.htm

HSV-8p in both strains — should have a similar relative effect in each strain. But, if breadth of the repertoire is important, depletion of Vβ8$^+$ T cells would affect the outcome differentially. When Vβ8$^+$ T cells were depleted, B6 mice showed enhanced mortality, whereas bm8 mice showed no difference in mortality compared with non-depleted mice. This provides evidence that CTL repertoire diversity can influence resistance to infection. In addition, CTLs from bm8 mice had higher avidity for antigen and were better able to kill target cells in a CTL assay.

The authors suggest that MHC-polymorphism-driven diversification of the TCR repertoire in bm8 mice allows high-avidity CTLs to be generated more readily, and that this might be the link between MHC polymorphism and resistance to infection.

Elaine Bell

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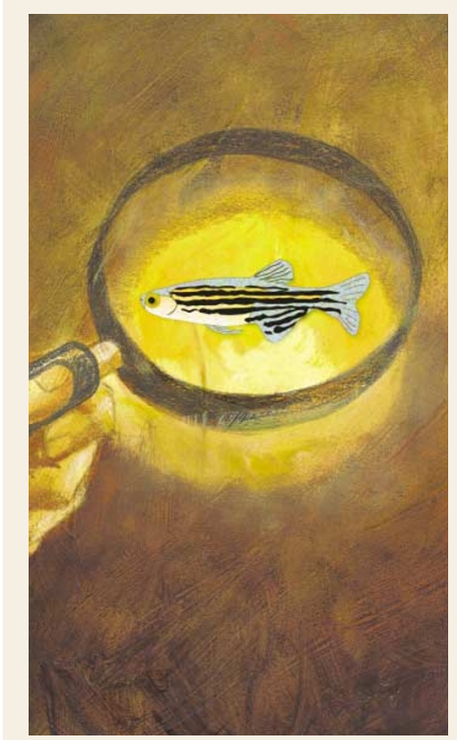
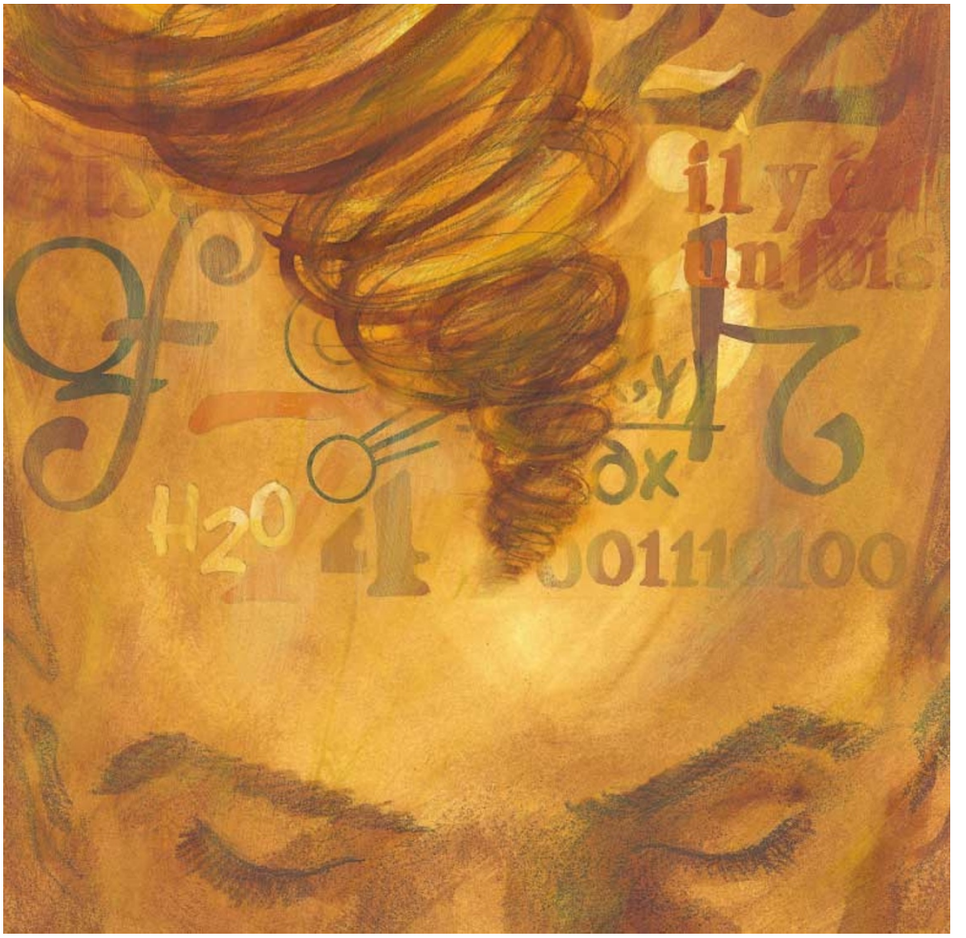
Janko Nikolich-Žugich's lab: http://www.ohsu.edu/vgti/zugich.htm





IMMUNOLOGICAL MEMORY

SAPping B-cell memory

Mutations of *SAP* (SH2 domain protein 1A) are responsible for X-linked lymphoproliferative disease, a syndrome that is characterized by three main phenotypes — fulminant infectious mononucleosis, B-cell lymphomas and dysgammaglobulinaemia. But, the precise role of SAP in controlling immune responses remains unclear. A new study, reported in *Nature*, shows that the expression of SAP by CD4$^+$ T cells is important for controlling the development of long-term humoral immunity.

B-cell responses were investigated in SAP-deficient mice after infection with lymphocytic choriomeningitis virus (LCMV). Wild-type mice generate strong primary responses, clear an infection with LCMV quickly and develop long-term specific B- and T-cell responses. SAP-deficient mice also make good primary responses, and they develop a similar number of virus-specific antibody-secreting cells (ASCs). When virus was cleared in the wild-type mice, the number of LCMV-specific ASCs decreased and then stabilized at about 10% of peak levels. But in SAP-deficient mice, ASCs were barely detectable by day 15. The number of long-lived plasma cells in the bone marrow was also much lower in SAP-deficient mice than in wild-type mice.

Next, the authors looked at the kinetics of the immunoglobulin-G response in the serum of SAP-deficient mice, and these were found to be comparable to the kinetics of ASCs. No significant differences in isotype switching were detected between wild-type and SAP-deficient mice. However, germinal centres were significantly reduced in both size and number in SAP-deficient mice, which indicates that germinal centres are necessary for the development of long-lived plasma cells.

So, which cell type is responsible for the defective development of long-lived plasma cells and of B-cell memory in SAP-deficient mice? To determine this, adoptive-transfer experiments were carried out. Mice that received SAP-deficient T cells and wild-type B cells followed by infection with LCMV had defective development of long-term humoral immunity. Further experiments showed that SAP must be expressed by CD4$^+$ T cells to enable long-term humoral immunity to develop.

This study has uncovered an unusual effect of a genetic defect, in which normal numbers of antigen-specific CD4$^+$ T cells are generated and early B-cell help occurs, but in which SAP deficiency is not permissive for the B-cell help that is necessary for the development of long-term humoral immunity.

Elaine Bell

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