Shn represses Brk transcription, which results in derepression of Dpp targets that are then subject to transcriptional activation by Mad-Med.

The TGF-β signaling pathway is conserved and elaborated in vertebrates (**Fig. 1b**) and contributes to many aspects of development[7,8]. There are over 30 TGF-β superfamily members in mammals, including TGF-β, BMP-2, BMP-4, nodal and activin, which bind to different combinations of the five type II and seven type I receptors (such as TβR-I, TβR-II, BMPR-I, BMPR-II, ActR-I and ActR-II). Combinatorial association of the type I and II receptors allows for similar or diverse responses to the numerous ligands, although in practice type I and II receptor combinations are limited. The activated type I receptor phosphorylates a receptor Smad (R-Smad) protein, which binds a common mediator Smad (Co-Smad) and migrates to the nucleus. The simplified version of this TGF-β signaling sequence is TGF-β binds to TβR-II and TβR-I, which results in the phosphorylation of Smad2. Activated Smad2 binds Smad4 and the complex moves to the nucleus. Smad proteins bind directly to DNA and also to numerous other transcription factors and other proteins[8]. Many of these proteins are important regulators of T cell gene expression, including β-catenin, T cell–specific factor 1 (TCF-1, also known as Lef-1), calmodulin, Jun, Fos, Sp1 and NF-κB.

Assuming that Shn-2 is part of a TGF-β superfamily pathway, the Shn-2 deficiency could affect positive selection through the intersection of such a pathway with the Ras–Erk MAPK pathway. Early work suggested that inhibition of this MAPK pathway showed a strong effect on positive selection without affecting negative selection, although more recent data show some effect on negative selection[3]. It has been suggested that sustained low-level signaling through the Ras-Erk pathway leads to positive selection, whereas a brief flurry of strong activity leads to negative selection[9]. Erk and other MAPKs are activated in response to TGF-β and BMP[8]. Also, the Ras-Erk pathway may regulate nuclear Smad activity, as Erk can inhibit nuclear translocation of R-Smads by phosphorylating them in a different manner from the TGF-β superfamily pathway[7]. In *Xenopus* and *Drosophila*, these pathways cooperate in cell fate determination[7].

Another potential point of interaction of a TGF-β superfamily pathway on positive selection could be through the Wnt signaling cascade. The TCF-1 transcription factor acts as a repressor in the absence of β-catenin. When β-catenin is stabilized as a result of Wnt signaling, it translocates to the nucleus and binds TCF-1, forming a transcription-activating complex. The interaction between TCF-1 and β-catenin is not required for positive selection *per se*, but is important in regulating the survival of immature DP cells long enough to allow them to rearrange TCRα and undergo positive selection efficiently[10]. TCF-1 binds TCRα and CD3ε enhancers and appears to regulate the anti-apoptotic protein Bcl-x_L[10]. TCF-1 binds to the R-Smads Smad-2 and Smad-3 as well as to the co-Smad Smad-4[11]; β-catenin also interacts with Smad-4[7,8]. Strikingly, the TGFβ pathway is able to independently activate TCF-1 target genes in *Xenopus*[11].

The effect of deletion of Shn-2 on thymocyte positive selection suggests that a TGF-β superfamily signal is important in positive selection. However, the potential role for Shn proteins in vertebrate TGF-β superfamily signaling is currently unknown. Assuming that one exists, it still remains to be determined how Shn-2 acts. Perhaps it is through a currently unknown vertebrate counterpart of Brk? It will also be important to determine how Shn-2 interacts with signals emanating from TCR signaling, and whether these are in fact related to the Erk, Wnt or other signaling pathways.

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Memory T cells: total recall or just a sense of *déjà vu*?

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Is a lifetime of sequential viral infections detrimental or advantageous to the host? New evidence suggests that pre-existing memory T cells specific for one type of virus can alter, for the better, the disease outcome after infection with an unrelated virus.

No one is naïve when it comes to infections. The immune system of any individual is shaped by lifelong and continuous exposure to antigens derived from foreign organisms. Those who use experimental models of viral infection tend to ignore this fact, although all experimentalists know that the hygiene of the mouse house can influence challenge experiments dramatically. In this issue of *Nature Immunology* Chen *et al.*[1] investigated whether infection with one virus can modify the way in which an unrelated virus is subsequently handled by the immune system. The results were unexpected.

Chen *et al.* first exposed mice intranasally to lymphocytic choriomeningitis virus (LCMV), an RNA virus that naturally infects the mouse. Infection with LCMV results in a vigorous $CD8^+$ T lymphocyte response, which is responsible for early antiviral control through perforin-mediated lysis of infected cells and release of interferon-γ (IFN-γ). After the virus is cleared, large populations of virus-specific T cells persist; these lymphocytes are rapidly effective at controlling a second challenge by the same virus. Once the LCMV-treated mice had cleared the virus, Chen *et al.* challenged them intranasally with vaccinia virus (VV), a presumably unrelated poxvirus. About 95% of these mice survived the challenge infection with VV, compared to only ~40% of naïve control mice. Titers of VV in lung, mediastinal lymph nodes (MLN) and spleen were 50%

lower at day 3 and 90–97% lower at day 6 after challenge in LCMV-immune mice compared to naïve controls, particularly if natural killer (NK) cells had been depleted before the challenge. In addition, adoptive transfer of splenocytes from LCMV-immune mice conferred a similar level of protection against intranasal VV challenge. This implies that there is also active recruitment of transferred cells to the site of VV replication. Notably, mice that showed a reduced VV titer had altered lung pathology. Whereas most naïve mice had severe alveolar edema and a mixed infiltration of polymorphonuclear and mononuclear cells, in LCMV-immune mice the lung infiltrate was largely dominated by lymphocytes and macrophages and there was much less alveolar edema.

Chen *et al.* showed that LCMV-specific memory cytotoxic T lymphocytes (CTLs) convey protection against heterologous virus challenge. Without any antigenic stimulation *in vitro*, ~20% of LCMV-specific CTLs within the lung infiltrates produced IFN-γ 3 days after VV challenge, but these cells had weak LCMV-specific *ex vivo* cytotoxic activity when tested 6 days after VV challenge. More notably, the protective effects of previous LCMV infection against VV challenge were abolished by the *in vivo* neutralization of IFN-γ. This IFN-γ secretion was not, however, confined to LCMV-specific cells, because somewhere between 40% and 75% of the activated $CD8^+$ T cells in the lung were non-LCMV–specific. In the range of 26–40% of $CD8^+$ T cells present in the lung were LCMV-specific when the VV challenge was given, and the proportion of LCMV-specific T cells did not change drastically upon VV challenge. It is thus not clear that LCMV-specific T cells were vital to the mechanism with which the immune system modulated the challenge. By analyzing this IFN-γ response for a series of epitopes, Chen *et al.* showed a shift in the immunodominant specificity of the responding LCMV-specific CTL populations after heterologous VV challenge, indicating that these antiviral cells did have some involvement in the enhanced protection that was seen.

How might exposure to one pathogen lead to enhanced resistance against another, unrelated, infection (**Fig. 1**)? A simple explanation is that T cell receptors (TCRs) of the first response recognize epitopes from both viruses. Chen *et al.* suggest that CTL epitopes of VV may cross-react with particular epitopes from LCMV (notably NP205), although the protection was not dependent on a particular peptide. To investigate this, it would be useful to establish whether the cross-reactive peptides act as full, partial or weak agonists in this system and whether a mutant LCMV lacking the NP205 epitope would confer the same level of protection against VV.

Other possible explanations relate to some form of 'bystander' effect. Non-TCR–mediated bystander activation and proliferation as well as maintenance of memory CTL frequencies are dependent on the effector cytokine interleukin 15 (IL-15)[2,3]. Two distinct pathways involved in bystander activation can induce IL-15: type I IFN, and IFN-γ in combination with IL-12 and IL-18[4]. Given that many pathogens (including VV) lead to substantial production of IFNs, it seems likely that these infections will induce bystander proliferation of memory CTLs. Where memory CTLs specific for a previous infection are present at very high frequencies (in LCMV, these comprise 20–30% of $CD8^+$ T cells in spleen and lung), either low-level cross-reactivity or cytokine-mediated bystander activation of even a small fraction of these cells will be detectable after heterologous challenge. Such high frequencies of memory CTLs are usually observed in persistent viral infections, which also influence the protective capacity of these cells[5]. Despite the presence of large numbers of antigen-specific $CD8^+$ T cells in—for example, human cytomegalovirus infection—there is no evidence that these cells protect against new infections. Although heterologous challenge leads to detectable functional activation of a fraction of some antigen-specific T cells, the effect is relatively small compared to that of a challenge with the cognate antigen.

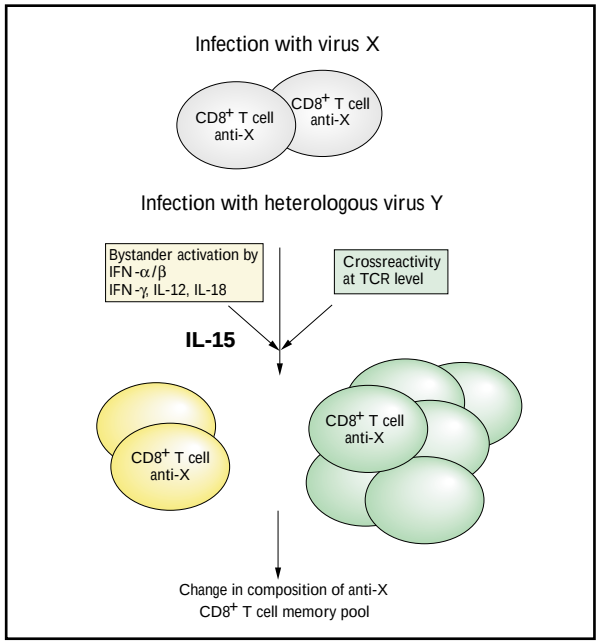

Figure 1. Memory $CD8^+$ T cells in heterogolous immunity. Memory $CD8^+$ T cells specific for virus X are confronted with infection by virus Y. Infection with heterologous virus Y triggers cytokine secretion (*via* target cell infection and activation of innate immune response). Bystander activation mediates memory T cell proliferation *via* the effector cytokine IL-15 and/or crossreactivity with virus X at the level of TCR recognition. A fraction of anti-X–specific memory $CD8^+$ T cells are activated, proliferate, redistribute between different organs and acquire direct *ex vivo* effector function, which can be asymmetric for different epitope specificities.

How general is this phenomenon likely to be? LCMV is unusual among viruses in that it induces very strong $CD8^+$ T cell responses that persist at high levels. This is why it is such a successful experimental model. The model is complicated, however. With some virus-host combinations, there is a tendency to persistence[6] and the immune response continues to evolve over a period of several weeks: for example, neutralizing antibodies take 6 weeks to emerge. During the memory phase, the overall proportion of $CD8^+$ T cells may increase by about 50% in the spleen and by two- to threefold in peripheral organs, such as the liver (U. Karrer and P. Klenerman, unpublished data). This suggests a profound long-term influence on the entire immune equilibrium of the mouse. It will be revealing to see to what extent the cross-protection shown by Chen *et al.* is seen in other viral systems, such as influenza, where there is no evidence of viral persistence.

How far can these animal experiments be extrapolated to human disease? The hepatitis G virus (GBV-C) is a persistent flavivirus that is common in patients with HIV but does not cause any significant disease. Unexpectedly, GBV-C infection appears to slow the progression of HIV[7,8]. Given that HIV is controlled by $CD8^+$ T cells, it is tempting to attribute this to the sort of immunological cross-protection explored by Chen *et al.*, although there are other plausible explanations, including virological interference. Notably, that finding contrasts with the accelerated HIV progression in those who also harbor hepatitis C virus. Indeed, when HIV patients are exposed to superinfecting pathogens, the outcome is often fatal (with the exception of scrub typhus which, oddly, is beneficial)[9].

Can bystander activation of T cells be potent enough to activate pre-existing autoreactive T cells and so cause tissue damage and clinical disease? This old concept has been addressed in the past with an experimental transgenic mouse model also involving LCMV and VV. High frequencies of CTLs (90% of $CD8^+$ T cells) specific for a neo-antigen expressed in pancreatic islets were insufficient to cause diabetes when they were activated in a "bystander-like" manner, despite substantial cytotoxic activity *ex vivo*[10]. These results suggest that unless bystander-induced CTL activation exceeds a critical threshold, which may be very high, organ damage does not occur.

As the host matures, diverse pathogenic challenges to the immune system could help to shape the composition of the T cell memory pool by causing proliferation[1] and "attrition"[11]. In all these experiments, it is also important to recognize that CTLs can be preferentially sequestered or "compartmentalized", confounding attempts to measure the total memory CTL pool[12].

Overall, the responses reported by Chen *et al.* are examples of the "fuzziness" evident in the logic of the immune system. Although the results these researchers found in manipulating the immune response do not represent classical memory, they resemble "memory-like effects", which may become apparent when multiple antigens are encountered sequentially. Perhaps what these antiviral T cells experience is not so much total recall as a case of *déjà vu*.

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Cytomegalovirus: from evasion to suppression?

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Viruses, such as CMV, have evolved a number of strategies with which to evade the immune system. Evidence is now emerging that murine CMV can also suppress the immune response by inducing functional paralysis of DCs.

Dendritic cells (DCs) are the professional antigen-presenting cells within the immune system and have emerged as key players in initiating the T cell response to viral infection[1]. This is, in part, related to their apparently unique capacity to prime naïve T cells and thus trigger differentiation of an activated effector T cell population. Uptake and processing of antigen is carried out by immature DCs that reside in peripheral tissues, where they express a panoply of receptors that allow them to respond to microbial products or pro-inflammatory stimuli. The recognition of such signals triggers the differentiation of immature DCs into mature DCs, which have a dramatically altered function. Mature DCs have a reduced capacity for antigen uptake, but express high amounts of major histocompatibility complex (MHC) as well as costimulatory molecules, which makes them excellent presenters of antigen to T cells. This maturation process is associated with altered expression of chemokine receptors that facilitates mature DC migration to the T cell areas of lymph nodes. The crucial role played by DCs in orchestrating the T cell response makes them an attractive and vulnerable target to viral attack. In this issue of *Nature Immunology*, Andrews and colleagues examine the consequences of murine cytomegalovirus (MCMV) infection on both *in vitro* and *in vivo* DC function[2]. They show that MCMV is able to both infect and induce a functional paralysis of murine DCs. This may represent yet another powerful mechanism by which MCMV manipulates the immune response.

Viral infection of DCs may be associated with two potential outcomes. Influenza infection causes DC maturation and the release of interleukin 12 (IL-12); this results in the induction of an extremely effective immune response[3]. However, an increasing number of viruses may actually impair antigen presentation, as has been reported for measles, HIV, vaccinia virus, dengue virus, venezuelan equine encephalitis virus, herpes simplex virus, smallpox and lymphocytic choriomeningitic virus (LCMV)[4]. Andrews and colleagues now show that MCMV firmly belongs within this latter grouping. As a herpesvirus, MCMV has been effectively exploited as a model system for human CMV (HCMV) infection and, indeed, persistent virus infections in general. However, caution should be exercised: one must not over-extrapolate from the MCMV animal model to HCMV disease because there are marked differences in the viral pathology of these infections and the degree of sequence similarity between the two genomes is surprisingly low[5]. HCMV has become a paradigm for viral immune evasion, and both HCMV and MCMV encode an elaborate array of immune-evasion strategies[6] (**Table 1**). Although it is clear that the viruses appear to adopt similar strategies, the mechanisms are not identical. This reflects the coevolution of each virus with its host: for example, HCMV and MCMV both encode multiple genes that interfere with MHC class I antigen presentation, but act at different stages in the pathway.

The specter now being raised is not merely that of immune evasion but of active suppression of the immune system mediated by impairment of DC function. Andrews and colleagues demonstrate productive infection of murine DCs with MCMV both *in vitro* and *ex vivo*. MCMV infection is associated with initial DC activation, followed by reduced expression of MHC as well as costimulatory molecules that are critical for T cell maturation. The infected DCs lose the capacity to secrete IL-12 or IL-2, which contributes to their functional paralysis. In addition, infected DCs remain unresponsive to additional maturation stimuli, such as lipopolysaccharide (LPS), and are unable to prime an effective T cell response. Therefore, prevention of the terminal stages of DC differentiation could result in inefficient presentation