NO ONE IS NAIVE: THE SIGNIFICANCE OF HETEROLOGOUS T-CELL IMMUNITY

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Memory T cells that are specific for one virus can become activated during infection with an unrelated heterologous virus, and might have roles in protective immunity and immunopathology. The course of each infection is influenced by the T-cell memory pool that has been laid down by a host's history of previous infections, and with each successive infection, T-cell memory to previously encountered agents is modified. Here, we discuss evidence from studies in mice and humans that shows the importance of this phenomenon in determining the outcome of infection.

T-HELPER TYPE 1/2 (T_H1/T_H2). At least two distinct subsets of activated $CD4^+$ T cells have been described. T_H1 cells produce IFN-γ, lymphotoxin and TNF, and support cell-mediated immunity. T_H2 cells produce IL-4, IL-5 and IL-13, support humoral immunity, and downregulate T_H1 responses.

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Infections with microorganisms can run markedly different courses in different individuals. This variability in pathogenesis has often been linked to the genetic make-up or physiological state of the host, but there is now an increasing body of work that indicates that previous exposure to related or, perhaps, unrelated infectious agents can greatly alter the host's immune response to an infection and cause a marked deviation in the disease course. We refer to this phenomenon as heterologous immunity; it might influence protective immunity, immunopathology, and/or the balance between T-HELPER TYPE 1 (T_H1) and T_H2 responses (immune deviation).

T-cell-mediated heterologous immunity and immunopathology might be common features in viral infections. The secondary challenge of a host with a virus might elicit only a modest T-cell response, because neutralizing antibody greatly restricts the replication of the challenge virus. However, the replication of a heterologous virus is not constrained by neutralizing antibody, and, if this heterologous virus can activate memory T cells that are specific for another previously encountered pathogen, the high antigen load might lead to profound T-cell activation. An example of this might be dengue fever, for which a severe shock syndrome can arise if a host that has been exposed previously to one of the four dengue-virus serotypes is later exposed to a second serotype[1,2]. We propose that altered immunopathogenesis might occur also with completely unrelated viruses, as a consequence of the activation of memory T cells. It has been known for a long time that residual effects of interferon (IFN) and activated macrophages might provide a level of heterologous immunity immediately after infection. Here, we discuss the effect of long-lasting memory T-cell populations on the pathogenesis that is induced by heterologous agents.

Modulation of the T-cell repertoire

$CD8^+$ T cells recognize processed peptides that are presented at the cell surface in the antigen-binding grooves of class I MHC proteins[3,4]. In general, the presented peptides are eight or nine amino acids in length, and they have distinct motifs that require two or three residues of the peptide to fit into pockets within the MHC groove[5]. Structural studies have indicated that the T-cell receptor (TCR) binds to the peptide–MHC complex by means of relatively few contacts with the peptide side chains that project out of the MHC groove[3]. Usually, for any one virus, there are many virus-encoded amino-acid sequences that have MHC-binding motifs, but the T-cell response is directed primarily against a small number of 'immunodominant' peptides. Immunodominance is influenced by the intracellular processing of the protein, the ability of the peptide to bind to an MHC molecule with high affinity and the available repertoire of TCRs that are able to recognize the peptide–MHC combination[4].

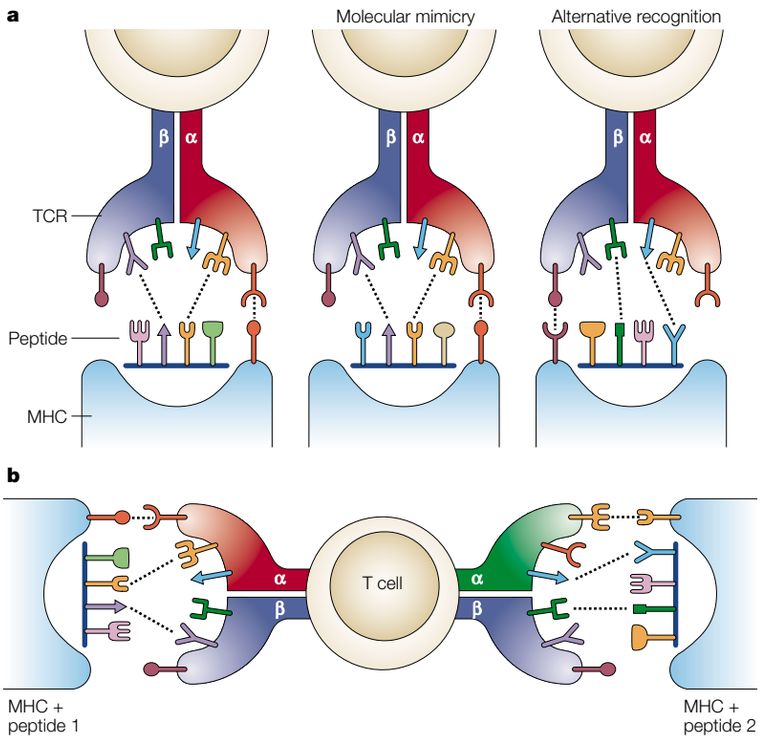

Figure 1 | **Potential mechanisms of T-cell crossreactivity. a** | The left-hand panel depicts the α- and β-chains of a T-cell receptor (TCR) interacting with a peptide that is presented by an MHC class I protein. The middle panel shows an alternative peptide that has similar determinants and interacts with the same TCR in the same manner as the first peptide. This is sometimes referred to as molecular mimicry[101]. The right-hand panel shows a situation in which different determinants of the TCR interact with the presented peptide[40,41]. We refer to this as alternative recognition. **b** | A single T cell might express two TCR α-chains, and the two distinct TCRs that are formed might recognize different antigens[42].

On encountering antigen under conditions of appropriate CO-STIMULATION, $CD8^+$ T cells undergo many cycles of division and differentiate into IFN-γ-producing cytotoxic T lymphocytes (CTLs)[6–8]. This proliferation and differentiation programme is predetermined and it can be initiated by only brief contact with the antigen-presenting cell (APC). Studies of 5,6-carboxy-fluorescein diacetate succinimidyl ester (CFSE)-labelled T cells that were transferred into mice indicate that a minimum of eight cell divisions occur after initial antigenic stimulation[6–8]. Calculations of limiting-dilution frequencies of antigen-specific T cells before (~1 in 100,000) and at the peak (~1 in 25) of the T-cell response — taking into account the 5–10-fold increase in the number of $CD8^+$ T cells — indicate that about 15 cell divisions occur[9]. Both techniques indicate that the generation time of these cells is only 6–8 hours. During this process of antigen-specific expansion, the virus is usually cleared, and the specific T cells decline in number either by apoptosis or by migration and dispersal into the non-lymphoid organs of the body, where they reside as memory T cells awaiting re-encounter with the stimulating antigen[10–12].

Carefully controlled experiments in mice have indicated that the hierarchy of the T-cell response to immunodominant peptides is consistent and predictable[4,13–18]. Hence, the specificity of the response is similar between genetically identical animals. Despite this, the TCR usage differs from animal to animal. Although there might be general similarities in preferred TCR Vβ usage, usually, the specific TCRs on the dominant T-cell clones are unique to the individual[19]. This is probably a consequence of several factors. First, the TCR repertoire of T cells that emigrate from the thymi of genetically identical mice is variable[20], because of the random stochastic process of TCR gene rearrangement. Second, the encounter of a T cell with an APC that displays the appropriate ligand is random. Third, those T-cell clones that are stimulated earliest might become 'dominating' clones and interfere with the stimulation of other T cells[4]. A host's unique antigen-specific TCR repertoire becomes fixed at the point of antigen clearance and, even though there is a marked reduction in the total number of antigen-specific T cells between the peak of the acute T-cell response and the memory state, the distribution of dominant T cells in the memory state remains proportionally similar to the distribution at the time when antigen was cleared[19,21].

Degeneracy of T-cell recognition. A TCR that recognizes a given MHC-presented peptide might also recognize other peptides that fit the appropriate MHC motif and have, projecting from the antigen-binding groove, amino-acid side chains that are able to stimulate the TCR (FIG. 1). In fact, it has been calculated, on the basis of positional analysis of various amino-acid substitutions at different residues of a peptide, that a given TCR has the potential to recognize a million different peptide–MHC combinations[22]. This result — as well as a substantial amount of experimental data that is discussed below — indicates that peptides do not necessarily need to have high sequence homology to be crossreactive with the same T cell. Moreover, memory T cells are in a physiological state that is primed for activation, and they can be productively stimulated by a peptide concentration that is 50 times lower than that required for the stimulation of naive T cells[23–27]. So, it would not be surprising if a memory T cell could be stimulated by a crossreactive peptide with substantially less affinity for the TCR than the original peptide that created the memory T-cell pool.

This issue of T-cell degeneracy was uncovered first by the analysis of T-cell clones that had unexpected crossreactivity — vesicular stomatitis virus (VSV)- and influenza-virus-specific CTL clones were shown to crossreact with uninfected allogeneic targets[28,29]; an influenza-virus nucleoprotein (NP)-specific clone was shown to lyse targets that were coated with an unrelated peptide derived from a different influenza-virus-encoded polymerase (PB2)[30]; and another influenza-virus matrix-protein-specific clone was shown to crossreact with a rotavirus VP4 peptide[31]. Studies of several virus infections in mice and of Epstein–Barr virus (EBV) infections in humans have shown that a high degree of allospecific CTL activity is generated during infection[32–36]. At first, this was attributed to non-specific, polyclonal BYSTANDER ACTIVATION, but limiting-dilution clonal assays have shown that much of this activity can be attributed to

CO-STIMULATION
Optimal signalling through the TCR complex requires accessory cell-surface molecules, such as CD28 or LFA1. Signals that are delivered from these molecules contribute to enhancing the immune response. In the absence of these co-stimulatory signals, naive T cells become unresponsive to a subsequent challenge with antigen.

CFSE
(5,6-carboxy-fluorescein diacetate succinimidyl ester). This a fluorescent dye that is used to label cells. With each cell division, the label is distributed equally into daughter cells. The loss of fluorescence intensity is used to calculate the number of cell divisions.

BYSTANDER ACTIVATION
The term, as it is used here, refers to the activation of T cells in which the TCRs are not being triggered by the antigens that are driving the immune response. This activation might be mediated by cytokines.

CLONAL IMPRINTING/ORIGINAL ANTIGENIC SIN
Previous exposure to one virus strain diverts the antibody response after exposure to a second virus strain to epitopes that are shared between the two strains.

BROMODEOXYURIDINE
(BrdU). A thymidine analogue that can be incorporated into DNA during S-phase when cells are exposed to this substance. Cells that have incorporated BrdU, and presumably have divided, can be visualized with anti-BrdU antibodies using flow cytometry.

T-cell clones that are crossreactive between virus-infected syngeneic targets and uninfected targets that express allogeneic MHC antigens[36–38]. CTLs that are specific for lymphocytic choriomeningitis virus (LCMV) were reactivated in LCMV-immune mice that were challenged with Pichinde virus, vaccinia virus or murine cytomegalovirus (MCMV)[39]. Again, this was speculated originally to be due to the polyclonal bystander activation of memory CTLs, but clonal analyses showed T-cell clones that are crossreactive between LCMV and Pichinde virus, and between LCMV and vaccinia virus[38].

Two structural studies that examined T-cell crossreactivity against allogeneic cells have shown that the same TCR can bind to different peptide–ligand structures[40,41] (FIG. 1). If different determinants on the TCR react with different peptide–MHC structures, it would be very difficult to predict when such crossreactivity would occur. By contrast, a crossreaction that involves the same determinants on the TCR might be easier to predict by searching for similar amino-acid side chains at positions of peptides that are accessible to the TCR; this method is used for the calculation of potential frequencies of crossreactivity[22]. A third structural explanation for crossreactivity that would be virtually impossible to predict would be if a given T cell expressed two different TCRs (FIG. 1b). This could happen as a result of incomplete allelic exclusion of the second TCR α-chain[42]. A further level of unpredictability is that it is probable that only a subpopulation of the T cells that are specific for a peptide will recognize the crossreactive peptide. Given that the TCR usage differs from host to host and that stochastic elements might determine clonal dominance[19], we can imagine that the proportion of peptide-specific T cells that crossreact with another peptide might differ from one host to another. Virus-induced T-cell crossreactivity with allogeneic targets might have significant implications for the maintenance of allogeneic transplants and for the shaping of the allospecific memory T-cell repertoire[35,43], but here, we are concerned about the potential relevance of crossreactivity between viruses, and how this shapes the $CD8^+$ T-cell memory pool and influences viral pathogenesis.

Immunodominance influenced by previous infection. Although immunodominance hierarchies for T-cell epitopes are consistent between genetically identical mice in controlled laboratory conditions, recent studies in HIV-1-infected patients have shown variability in the hierarchies of known HLA-A2-restricted epitopes[44,45]. We propose that one factor that might regulate immunodominance in this uncontrolled 'wild' human population is previous exposure to other pathogens, which might have altered the hierarchy of the T-cell repertoire. For example, Brehm *et al.*[104] have shown that LCMV and Pichinde virus encode crossreactive $CD8^+$ T-cell epitopes that have six out of eight amino acids in common. These peptides are subdominant in each infection; they elicit T-cell responses that account for less than 3% of the antigen-specific $CD8^+$ T cells during acute infection and about 1% of the $CD8^+$ T cells in the memory pool. If LCMV-immune mice are infected with Pichinde virus, or if Pichinde-virus-immune mice are infected with LCMV, the T-cell responses to these peptides become dominant, involving more than 20% of the $CD8^+$ T cells. Hence, infections with heterologous agents can affect immunodominance when crossreactive peptides are present (FIG. 2). This is similar to the concept of CLONAL IMPRINTING/ORIGINAL ANTIGENIC SIN that was proposed initially to explain the anamnestic antibody response to crossreactive B-cell epitopes of related influenza-virus strains[46] and that was used more recently to describe the crossreactive T-cell responses to variants of the same viruses[47,48]. This brings in to question any studies of immunodominance hierarchies in response to human viral infections, as we have no idea how previous infections have influenced these hierarchies.

Bystander activation of memory T cells. Memory $CD8^+$ T cells are able to respond to stimuli in a bystander manner in the apparent absence of TCR ligation[49,50]. However, it is difficult to exclude a role for TCR stimulation, because all TCRs have some low level of reactivity against endogenous ligands, the expression of which might be upregulated by virus-induced cytokines. It is also difficult to be certain when T cells are responding as a result of bystander mechanisms or crossreactive stimulation with viral peptides. Experiments that are designed to rule out crossreactivity against viral peptides indicate that T-cell populations that are not specific for the virus do not increase in number in the spleen during viral infections, and, if anything, they might decrease in number[51,52]. But, this does not negate the possibility that the non-specific T cells experience some level of activation.

Most of the evidence seems to indicate that memory T cells, which express distinct chemokine receptors[53], migrate into areas of inflammation in a non-specific manner. This increases the probability that a memory T cell will encounter its ligand. Recent studies have shown that putatively non-crossreactive ovalbumin (OVA)-specific, naive TCR-transgenic T cells are not attracted to the influenza-infected lung, whereas memory-phenotype, OVA-specific transgenic T cells migrate into the lung early during influenza-virus infection and thereafter disappear instead of expanding in number[54].

Memory $CD8^+$ T cells have receptors for interleukin-15 (IL-15), a cytokine that seems to regulate their homeostasis[50,55]. During viral infection, there is an induction of expression of type I interferon, which can then induce the expression of IL-15 by macrophages and dendritic cells. In turn, IL-15 can enhance the division of memory $CD8^+$ T cells, as shown by the uptake of BROMODEOXYURIDINE (BrdU) *in vivo*[49,52,55]. This division tends to be a homeostatic process, and it does not lead to a significant increase in the total number of $CD8^+$ T cells[52]. In fact, homeostatic division might be necessary during the virus-induced IFN response because type I IFN induces the apoptosis of memory $CD8^+$ T cells[52]. Stimulation of mice with the type-I-IFN inducer poly inosinic/cytidylic acid (poly I:C) induces first a substantial loss (> 50%) of memory $CD8^+$ T cells. Then, it seems that IL-15 stimulates the division of the

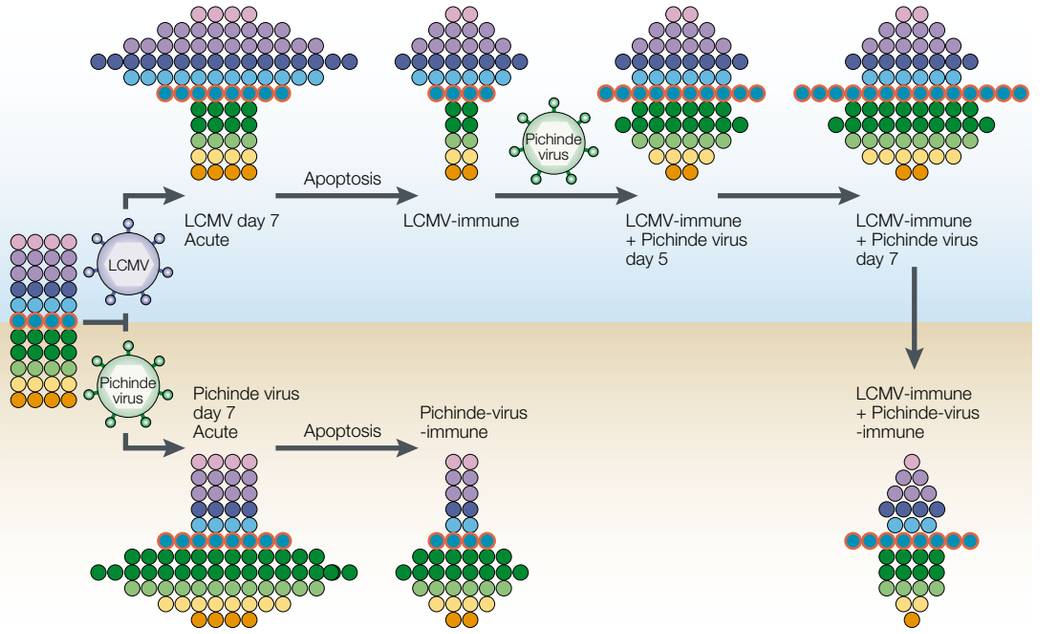

Figure 2 | **Modulation of the T-cell repertoire during viral infection.** The coloured dots represent T-cell populations that have different specificities. Here, a naive immune system is challenged with either of two heterologous viruses — lymphocytic choriomeningitis virus (LCMV) or Pichinde virus. Some of the T-cell populations expand to combat the infection and then undergo apoptosis, which leaves the host with a skewed memory T-cell pool. If an immune system that has been conditioned by one virus infection (LCMV) is exposed to another virus (Pichinde virus), T-cell populations that are crossreactive with the two viruses (red outline) will expand preferentially and dominate the response. After the response, memory T cells that are specific for the first virus only are reduced in number, whereas the crossreactive T cells are preserved and enriched in the resting memory pool. Adapted, with permission, from REF. 102 © (1995) Hogrefe & Huber.

remaining $CD8^+$ T cells, such that they restore the $CD8^+$ T-cell pool[52]. Of course, these $CD8^+$ T-cell population dynamics change during a viral infection, when activated virus-specific T cells will be competing with the resting memory T-cell pool.

Recent BrdU-labelling studies in influenza-virus- and mouse γ-herpesvirus-infected mice have indicated that antigen-specific T cells cycle much more rapidly than bystander T cells[56–58]. In addition, a comparison of two influenza-virus strains that encode closely related T-cell epitopes (using MHC–peptide tetramers to identify antigen-specific T cells) showed that there is a substantially greater proliferation of T cells that are crossreactive with the challenge-virus epitope than of T cells that are specific only for the previously encountered virus[47]. It remains unclear if non-specifically stimulated cells, which do not seem to increase substantially in number, have important antiviral effector functions.

Fate of memory T cells during sequential viral infections. In the absence of antigenic stimulation, $CD8^+$ T-cell memory is remarkably stable in a host that has previously experienced a viral infection[9,59,60]. By undefined mechanisms that might involve IL-15 and internal T-cell biological clocks, memory $CD8^+$ T cells divide occasionally and maintain their cell numbers over a period of many months[50,61–63]. The number of these memory T cells can be quite high. For example, one year after an LCMV infection, about 15% of spleen $CD8^+$ T cells were LCMV-specific, and even higher frequencies were seen in peripheral organs[64,65]. This stable, high-frequency response is disrupted by infection with heterologous viruses, which leads to quantitative reductions in memory $CD8^+$ T cells that are specific for previously encountered pathogens[9,64]. This loss in the long-term memory T-cell pool is consistent with studies that have shown that memory $CD8^+$ T cells that are specific for agents other than the infecting virus undergo apoptosis and decline in frequency during acute infections[52]. An exception to this phenomenon occurs when there are crossreactive epitopes between the heterologous viruses; T cells that recognize crossreactive epitopes are preserved and might be enriched in the memory population[104]. So, homeostasis of $CD8^+$ memory T-cell pools is maintained by two mechanisms: the loss of non-crossreactive T cells and the preservation of crossreactive T cells (FIG. 2). We do not mean to imply that antigen, be it crossreactive or otherwise, is required to maintain memory $CD8^+$ T cells, but that crossreactive antigen will offset the non-specific deletion of memory T cells (attrition) that occurs during new infections.

For reasons that are not well understood, the dynamics of $CD4^+$ T-cell responses are different from those of $CD8^+$ T-cell responses. There is a less dramatic increase in the number of virus-specific $CD4^+$ T cells during the acute response, a more dramatic loss between the peak of the acute response and the memory phase, and a gradual erosion of the memory $CD4^+$ T-cell response

with time[60,66,67]. Crossreactive CD4+ T-cell responses between heterologous viruses have not been examined systematically. It is of interest, however, that heterologous viral infections do not accelerate the decline in CD4+ T-cell memory, perhaps because relatively few CD4+ T cells enter the memory pool and compete with the resident population[68].

Heterologous immunity and immunopathology

The question arises as to whether the observed modulations of memory T cells that are specific for previously encountered agents will alter the pathogenesis of subsequent infections with unrelated heterologous viruses. Several recent experiments indicate that this is indeed the case, and these alterations can result in protective immunity, altered immunopathology and/or changes in the T_H1/T_H2 balance (immune deviation).

Protective immunity. Protective heterologous immunity between unrelated viruses was shown by 'checkerboard' analyses, in which mice that were immune to one of several viruses — LCMV, Pichinde virus, vaccinia virus or MCMV — were challenged with other viruses (FIG. 3). The results showed many instances of partially protective, but not necessarily reciprocal, immunity[69]. Infection with LCMV, Pichinde virus or MCMV conferred a considerable level of protection against infection with vaccinia virus, as shown by reductions in viral titres and increased survival in response to lethal doses of vaccinia virus in systemic and respiratory-mucosal models of infection[65,69]. Of interest, the heterologous immunity against vaccinia virus was not reciprocal, as vaccinia-virus-immune mice did not have resistance to any of the other viruses. Similarly, LCMV protected against Pichinde virus better than Pichinde virus protected against LCMV. In general, protected mice had a 2–200-fold reduced viral titre 3–4 days after infection compared with the challenge of immunologically naive mice, and the protection continued for as long as a year after the primary virus infection[69,70]. This heterologous protection was significant, although it was considerably less than the almost total protection that is seen after challenge with a homologous virus. The lack of reciprocal protection that sometimes occurs between heterologous viruses might relate to whether the potentially crossreactive T-cell epitope is sufficiently dominant to generate a sizeable pool of memory T cells. If the frequency of crossreactive T cells is relatively high after the first viral infection, then protective immunity might restrict the replication of the second virus. If the frequency of crossreactive T cells is very low after the initial infection, then the protective immunity might be weak. We might predict that large viruses, such as vaccinia virus and MCMV, encode many peptides that are able to stimulate some of the T cells in a pre-existing memory pool. That might, in part, be why so much of the genetic information of large DNA viruses encodes proteins that interfere with antigen presentation or are involved in other forms of immune evasion[71]. We could also speculate that viruses that have very small genomes might escape surveillance by heterologous memory T cells, and it is noteworthy that many small RNA viruses, such as Ebola, Lassa, Hanta and yellow-fever viruses can cause rapidly progressing and fatal diseases.

Challenge virus

Immunizing virus	LCMV	PV	MCMV	VV
LCMV	ND	+	+/–	++
PV	+/–	ND	+	++
MCMV	+	+/–	ND	++
VV	–	–	–	ND

Figure 3 | **Protective heterologous immunity between viruses.** Naive mice or mice that were immune to various heterologous viruses were challenged with these viruses, and the titres of plaque-forming units were assessed in organs 3–4 days after infection. This figure — which is based on data from REF. 69 — shows the degree of protective immunity, as determined by the reduction of viral titre, in heterologous-virus-immune mice compared with naive mice. Homologous virus challenges were not assessed. LCMV, lymphocytic choriomeningitis virus; MCMV, murine cytomegalovirus; ND, not determined; PV, Pichinde virus; VV, vaccinia virus; –, no change in titre; +/–, 2–5 times reduced titre; +, ~10 times reduced titre; ++, ~100 times reduced titre.

The mechanisms that underlie heterologous immunity have been investigated in LCMV-immune mice that were challenged with either Pichinde virus or vaccinia virus. Both viruses recruited LCMV-specific memory T cells to the site of infection — whether it was the lung or the peritoneal cavity — and both induced the reactivation of cytolytic function and skewed proliferation of subpopulations of LCMV-specific CD8+ T cells. Adoptive-transfer studies indicated that protection against either virus was mediated by a combination of CD4+ and CD8+ T cells from LCMV-immune mice[65,69]. Mechanistic studies indicated a strong role for IFN-γ in protection against vaccinia virus but not against Pichinde virus (FIG. 4). Vaccinia virus — which is very sensitive to IFN-γ — induced the *in vivo* production of IFN-γ by LCMV-specific CD8+ T cells by 3–4 days after infection, and heterologous immunity against vaccinia virus did not occur in mice that lacked IFN-γ responses[38,65]. Infection with Pichinde virus induced relatively low levels of IFN-γ in LCMV-immune mice and the virus seemed to be controlled by a different mechanism, such as cytotoxicity[69]. Both vaccinia virus and Pichinde virus induced the preferential expansion of discrete populations of LCMV-specific T cells, which indicates that crossreactive CTLs might have a role in heterologous immunity, and crossreactive epitopes between vaccinia virus/Pichinde virus and LCMV have been identified recently[65,104]. These results indicate that some combination of crossreactive

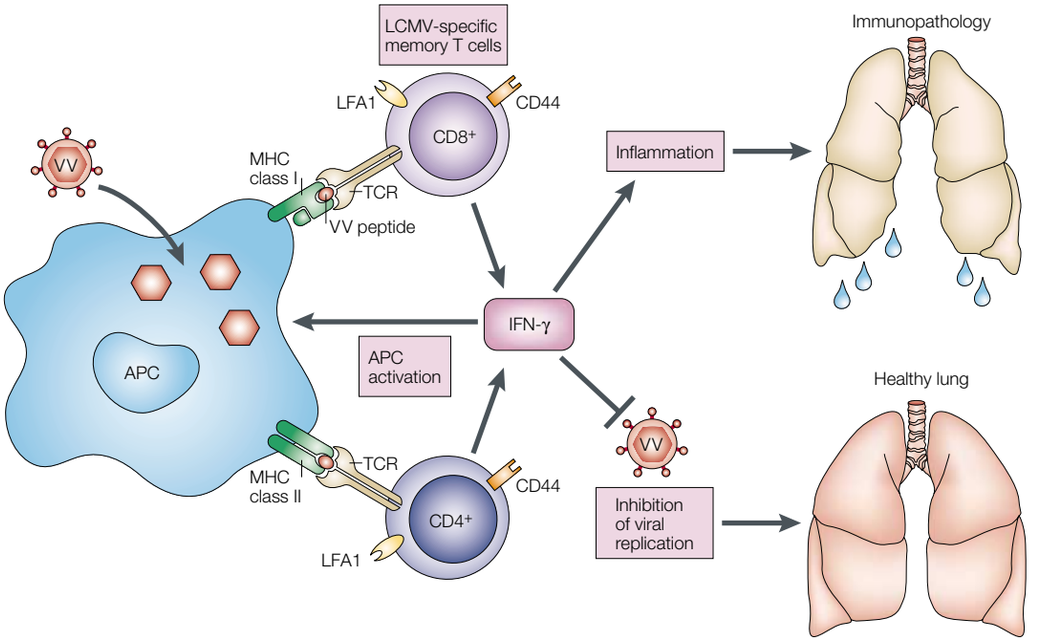

Figure 4 | **Model of heterologous immunity in the lung.** Some of the memory T cells that are specific for one virus (lymphocytic choriomeningitis virus; LCMV) are crossreactively stimulated by antigens from a second heterologous virus (vaccinia virus; VV). This causes the release of interferon-γ (IFN-γ), which further activates antigen-presenting cells (APCs) and enhances their expression of MHC molecules. Together, these events antagonize viral replication and, at the same time, facilitate the development of immunopathological lesions, perhaps in part through the release of tumour-necrosis factor and other inflammatory cytokines. Lymphocyte function-associated antigen 1 (LFA1) is an adhesion molecule, the expression of which is upregulated on memory T cells. CD44 is a memory-cell phenotypic marker. TCR, T-cell receptor.

T-cell triggering and the cytokine milieu alters the outcome of an acute virus infection in a host that has previously been exposed to another viral pathogen[65]. The inference from these results in experimental models is that heterologous immunity in humans might be the determining factor between a clinical and subclinical, or between a lethal and non-lethal, infection. This concept has not received sufficient study in humans so far, but it is noteworthy that in developing countries, live measles-virus vaccine, but not diphtheria–tetanus–pertussis vaccine, seems to protect against mortality that is not attributed to measles-virus infection[72].

Altered immunopathology. A heterologous virus has the potential to be a strong stimulator of memory T cells that are specific for another virus because its replication would be unimpeded by neutralizing antibodies, which would rapidly clear the virus. The most extreme example of this might be sequential infections in humans with different strains of dengue virus that express distinct neutralizing-antibody epitopes but that share highly homologous T-cell epitopes. This could lead to a very potent T-cell response that some have hypothesized might be responsible for dengue shock syndrome[1,2]. What role do memory T-cell responses have in human influenza-virus infections? Influenza variants can become pathogenic to an immune human population after the viruses develop mutations in, or reassortments of, their haemagglutinin gene, which makes them resistant to antibody-mediated neutralization[73,74].

Experimental models have shown that a history of unrelated viral infections can greatly influence immunopathology[65,69,75]. During intraperitoneal infections, LCMV-immune mice that were challenged with vaccinia virus developed severe immunopathological lesions in visceral fat[69,75]. These lesions were characterized by infiltrates of T cells and macrophages, and large areas of necrosis (FIG. 5A,B). This acute fatty necrosis was analogous to human panniculitis — the most common presentation being erythema nodosum (FIG. 5C) — although similar visceral necrosis occurs in systemic lupus erythematosus[76]. Intranasal infection of LCMV-immune mice with vaccinia virus resulted in a markedly altered lung pathology compared with non-immune mice that were infected with vaccinia virus[65]. The vaccinia-virus-infected non-immune mice developed pulmonary oedema, which resulted in the filling of air spaces. Presumably, this reduced gaseous exchange and might have been the cause of the increased mortality of these mice at higher doses of virus. By contrast, the vaccinia-virus-infected lungs of LCMV-immune mice had a dramatic expansion of the normally insignificant lymphoid compartment and the bronchus-associated lymphoid tissue (BALT), and this was infiltrated with LCMV-specific $CD8^+$ T cells. The presence of these activated T cells might have contributed to the development of bronchiolitis obliterans — an obstruction of the bronchiole by plugs of fibrin and inflammatory cells[70] — in some mice (FIG. 5D,E). In humans, the aetiology of this condition is not well understood, much like that of

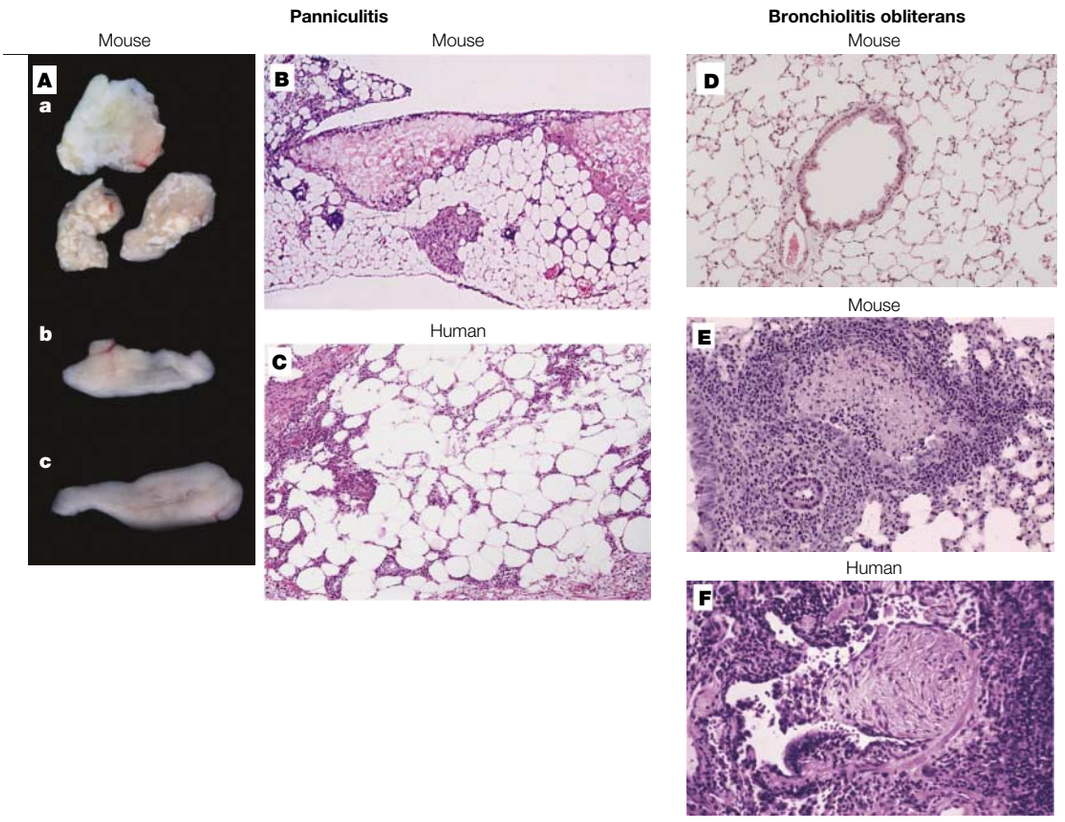

Figure 5 | **Comparison of pathology in fat and lung in models of heterologous immunity in mice, and in human diseases of unknown aetiology. A** | Specimens of visceral fat that show (**a**) necrosis in lymphocytic choriomeningitis virus (LCMV)-immune mice that have been infected intraperitoneally for five days with vaccinia virus, compared with normal-looking fat from (**b**) a naive mouse that has been challenged with vaccinia virus and (**c**) an unchallenged LCMV-immune mouse. **B** | Histology of visceral fat with areas of necrosis and mononuclear-cell infiltrates (panniculitis) from a vaccinia-virus-infected LCMV-immune mouse. **A** and **B** are based on work that is published in REFS 69,75. **C** | Similar features can be seen in human skin from a patient with erythema nodosum, a form of panniculitis (courtesy of Bruce Smoller)[103]. **D** | A control mouse strain that shows no inflammation and an open airway. **E**, **F** | Histology of bronchiolitis obliterans, which shows fibrous occlusion and the partial destruction of the airway with mononuclear infiltrates. These features are seen (**E**) in the lung of an LCMV-immune mouse seven days after vaccinia-virus infection (reproduced, with permission, from REF. 65 © (2001) Macmillan Magazines Ltd.) and (**F**) in a human lung (courtesy of Armando Fraire).

erythema nodosum. Both of these human diseases are thought to be immune-mediated and to occur in association with viral and intracellular bacterial infections. These experimental models indicate that potent T_H1 responses have an important role in immunopathology, as the lesions in both fat and lungs were dependent on the production of IFN-γ in the vaccinia-virus-infected LCMV-immune mice[65,69] (FIG. 4). These models indicate clearly that an individual's past history of infection might influence the immunopathology that develops on encounter with another infectious agent.

In addition, viral infections have been linked to the induction of autoimmunity[77], and it is possible that heterologous immunity might be a contributing factor. For example, mice that express an LCMV *NP* transgene in the brain develop transient encephalitis after infection with LCMV but not with Pichinde virus or vaccinia virus[78]; however, after LCMV had broken tolerance and elicited a memory T-cell response that was specific for the 'self' NP antigen, subsequent infections with Pichinde virus or vaccinia virus were able to reactivate some LCMV-specific T cells and re-elicit the disease. Hence, heterologous virus infections can result in exacerbations and remissions of autoimmune conditions, which is somewhat analogous to the course of multiple sclerosis.

Immune deviation. Naive transgenic T cells can be induced to differentiate in a T_H1 or T_H2 direction by different concentrations of antigen or by exposure to cytokines that are produced by T_H1 or T_H2 cells, respectively[79–81]. If a pre-existing pool of memory T cells is activated during infection with a heterologous agent, the T_H1 or T_H2 bias of the memory response might affect the T_H1 or T_H2 bias of the primary response to the heterologous agent. For example, infection with a virus such as LCMV might leave the host with a large memory T-cell pool that is biased towards T_H1-type responses, and if these cells become reactivated, the IFN-γ that they produce might orient the next response into the T_H1 pathway[67]. Certainly, there are unusually high levels of IFN-γ produced in LCMV-immune mice

that are challenged with vaccinia virus[65]. By the same argument, an immunization that leaves the memory T-cell pool with a T_H2 bias might orient a subsequent response into the T_H2 pathway. This could be problematic, as T_H1 responses are important for the control of several viral and intracellular bacterial infections, whereas T_H2 responses have been associated with viral persistence, aberrant pathology and allergy[82–84]. It is noteworthy that many vaccinations induce T_H2-like responses, even though under natural conditions of infection, the induction of a T_H1 response would be preferable to control the infection[83].

The evidence that such immune deviation actually takes place is limited but intriguing. In the 1960s, a formalin-inactivated respiratory syncytial virus (RSV) vaccine was introduced. Many of the vaccinated individuals had poor protective immunity to RSV challenge and developed, instead, unusually severe symptoms that were associated with profound lung eosinophilia — which is now known to be a potential consequence of IL-5 production during a strong T_H2 response[82,85,86]. This homologous system indicates that an inappropriately formed memory T-cell pool might lead to an aberrant response. Several groups have now developed models in which this type of pathology can be mimicked in mice by including heterologous viruses in the immunization process[84,87–89]. Immunization of mice with a vaccinia-virus recombinant that expresses the RSV G-protein primes for, on RSV challenge, an aberrant response that is associated with lung eosinophil infiltrates, T_H2 cytokines and a very narrow, Vβ14-restricted, damaging T-cell response. It is of interest that if mice are infected with influenza virus before the vaccinia-virus–RSV immunization and RSV challenge, the immune response to RSV is altered and the infection resolves quickly without serious eosinophilia[88].

Epidemiological data have indicated that the incidence of allergies is much higher in developed countries than in the developing world. This might be due to improved hygiene and the vaccinations that children in developed countries receive. In addition, children in underdeveloped countries might experience a series of infections that mould their immune systems differently than those of less-exposed children[83,90–92]. We propose that the imprinting of memory T cells to the T_H1 phenotype by previous exposure to pathogens might mould the immune system in a positive way, enabling a more effective response against subsequently encountered pathogens and, possibly, inhibiting allergic responses. Epidemiological evidence indicates that humans that are immunized against *Mycobacterium tuberculosis* bacillus Calmette–Guerin (BCG) — a strong T-cell and IFN-γ stimulus — might have a lower rate of atopic disorders[83,90]. In support of this concept, mice that were immunized with BCG had a suppressed T_H2 response and considerably reduced lung eosinophilia when exposed to an allergen[93].

New hints of heterologous immunity. Recent analyses of the specificity of human T-cell responses have shown the potential for heterologous immunity in important human infections. For example, an immunodominant, HLA-A2-restricted T-cell epitope that is encoded by hepatitis C virus (HCV NS3-1073; CVNGVCWTV) has seven out of nine amino acids in common with an influenza-virus immunodominant peptide (NA-231; CVNGSCFTV), and T cells crossreact with the two epitopes[94]. So, a history of influenza-virus infection might confer a level of resistance to HCV, and it is noteworthy that some patients clear HCV, but others, for unknown reasons, develop persistent infections.

What has been apparent for many years is that many viral infections, such as with varicella zoster (chickenpox), measles, mumps and Epstein–Barr viruses, are far more symptomatic in teenagers and young adults than they are in young children[95,96]. Could this be due to the activation of memory T cells that are specific for previously encountered ubiquitous pathogens? Teenagers and young adults tend to develop more-pronounced immunopathological lesions than immunologically less mature children. Pronounced T-cell responses are, in fact, the characteristic feature of EBV-induced mononucleosis (which involves an expansion of the number of T cells)[97]. Work in our laboratory has indicated that some T cells that are specific for the main HLA-A2-restricted immunodominant peptide of EBV ($BMLF1_{280–288}$; GLCTLVAML) crossreact with the main HLA-A2-restricted immunodominant peptide of influenza virus ($M1_{58–66}$; GILGFVFTL), even though the peptides have only three amino acids in common. Does a strong presence of influenza-virus-induced M1-specific T cells in the memory T-cell pool predispose the host to severe mononucleosis on EBV infection? Such crossreactive T cells might also provide enhanced resistance to infection.

The types of crossreactive T-cell response that are listed above would indicate that hosts that have never experienced a particular pathogen might, nevertheless, have memory T-cell pools that are specific for it by virtue of crossreactivity. The discovery of the epitope that is crossreactive between HCV and influenza virus was, in fact, made when individuals that were seronegative for HCV were found to generate a putative 'HCV-specific' T-cell response[94]. Such a phenomenon could relate to recent findings of HIV-specific T cells in HIV-seronegative individuals who show no signs of harbouring HIV[98]. Several HIV-exposed persistently seronegative individuals have low-level HIV-specific T-cell responses to epitopes that are different from those that are recognized by HIV-infected seropositive individuals in the same community. Could the T-cell responses in the HIV-resistant subjects be the result of memory T cells that are crossreactive with other pathogens, and could those crossreactive responses confer a state of immunity? Of interest is the very recent observation that HIV-infected patients that are co-infected with the GBV-C flavivirus tend not to progress to AIDS[99,100]. Could this be an important example of heterologous immunity?

Concluding remarks

The field of heterologous immunity is in its infancy, but we suspect that the more investigators look, the more

examples they will find of T-cell crossreactivities between heterologous viruses. Given that such cross-reactivities have been shown to modulate the course of viral infection in animal models, focus should now be put on understanding how these modulations of pre-existing memory T-cell pools influence the pathogenesis of human diseases. We propose that pre-existing memory T cells have roles in many human infections, as no one more than a few weeks old is immunologically naive. An experienced immune system is likely to incorporate the easily activated memory T cells into defence against pathogens that have not been encountered previously. Future work should include studies to further determine the structural basis of crossreactivity and a more in-depth examination of cross-reactivity within the $CD4^+$ T-cell population.

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