Private aspects of heterologous immunity

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Clinical manifestations of viral infections are highly variable, both in type and severity, among individual patients. Differences in host genetics and in dose and route of infection contribute to this variability but do not fully explain it. New studies now show that each subject's history of past infections individualizes the memory T cell pool. Private T cell receptor specificities of these preexisting memory T cell populations influence both disease severity and outcome of subsequent, unrelated virus infections.

Heterologous immunity is the term used to describe the phenomenon by which memory T cells that were generated during an earlier infection are reactivated in response to a second, unrelated infection. This phenomenon was originally identified and characterized in a series of well-controlled studies of viral infections in mice (1–4). In this issue, a study by Urbani et al. illustrates the potential significance of heterologous immunity in the pathogenesis of human viral infections (5). The authors studied hepatitis C virus (HCV) infection, a disease that affects more than 170 million people worldwide. Typically, the onset of HCV infection is asymptomatic, and persistent infection develops despite the presence of a CD8 T cell response (6, 7). Urbani et al. describe two patients with a very rare fulminant onset of HCV infection (5). Both patients displayed an unusual CD8 T cell response that was unprecedented in its strength and narrow focus. About 36% and 12% of all peripheral blood CD8 T cells from these respective patients targeted a single epitope within the HCV nonstructural protein 3 (NS3) and also cross-reactively recognized an influenza A virus neuraminidase epitope with close sequence similarity (5, 8). In contrast, patients with nonfulminant onset of HCV infection displayed a broader, multispecific CD8 T cell response of lower magnitude (5–7, 9). The authors concluded that exposure to influenza A virus, as confirmed by cellular immune responses against a second influenza A virus epitope, preconditioned the CD8 T cell response to HCV and focused it on a single cross-reactive epitope. The result was severe immunopathology. The notion of cross-reactivity was supported by the demonstration that only those T cells that bound HCV NS3 epitope–MHC tetramers produced interferon-γ and increased cell surface expression of the degranulation marker CD107a in response to stimulation with the cross-reactive influenza A virus epitope. In contrast, no response was observed upon stimulation with an unrelated, noncross-reactive influenza A virus epitope. The cross-reactive nature of the response was further confirmed by the demonstration that those T cells that did not bind HCV NS3 epitope–MHC tetramers did not respond to stimulation with the cross-reactive influenza A virus epitope.

Although the study did not provide direct evidence that preexisting influenza A virus–specific memory T cells were indeed the source of the rapid, vigorous, and narrowly focused HCV-specific T cell response, this is a plausible scenario for two reasons. HCV–cross-reactive T cells can be induced by influenza A virus infection of HLA-transgenic mice (8), and HCV/influenza cross-reactive cells have also been described in healthy blood donors, who are not HCV infected and have no history of HCV infection (8).

Factors determining cross-reactive immune responses

Two factors that determine the frequency and extent of T cell cross-reactivity are the likelihood of exposure to a given virus and the sequence variability of that virus. Influenza A virus infection is one of the most common viral infections in humans. Variant strains of influenza are abundant, and reexposure to the virus is common. If it can be assumed that most HCV-infected people have been infected previously with influenza A virus, why isn't cross-reactivity and severe immunopathology a more common feature of HCV infection? In fact, despite the high prevalence of influenza A virus infection, fulminant acute hepatitis C is a very rare event (10, 11). This may be due to several factors. First, the HLA haplotype of the patient is important. In the study by Urbani et al., the cross-reactivity between the HCV NS3 epitope and the influenza A virus neuraminidase epitope was confined to HLA-A2–positive patients, as both epitopes are HLA-A2 restricted (5, 8). Second, the sequence diversity of HCV genomes that coexist in each patient and the high mutation rate of HCV may allow the virus to escape from the T cell response and prevent the reactivation of cross-reactive T cells and the induction of immunopathology. In the case of the HCV NS3 epitope, mutations have been identified both within and outside the epitope (12, 13). Mutations within the epitope may generate a T cell receptor antagonist (12), and a mutation in its COOH-terminal flanking sequence has been shown to affect proteasomal processing of the epitope and to reduce the induction of epitope-specific T cells (13). If the infecting virus displays these mutations in an HLA-A2–positive patient, the altered HCV NS3 sequence may not sufficiently activate preexisting cross-reactive memory T cells. These factors are difficult to assess in human studies because patients typically seek medical attention late after the actual infection or not at all, making it impossible to determine the sequence of the

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original, infecting virus and to define the patient's early immune response.

An additional layer of complexity is described in the elegant study of Kim et al. in the previous issue of the *JEM* (14). Using a murine model of sequential lymphocytic choriomeningitis (LCMV) and vaccinia virus (VV) infections, the authors report differences in T cell cross-reactivity even among mice of identical genetic background that were infected with identical viruses. When mice were infected with LCMV, they displayed similar hierarchies in the epitopes recognized but differed in their TCR usage. These differences in TCR CDR3 sequences determine the private specificity of epitope-specific T cell responses. When genetically identical, LCMV-immune mice were then challenged with the unrelated VV, the pattern of cross-reactivity varied between mice. In contrast, when memory CD8 T cells from a single LCMV-immune mouse were adoptively transferred into several recipient mice, each of the recipient mice mounted nearly identical responses to the subsequent VV infection. The CD8 T cell response to VV challenge was influenced by preexisting cross-reactive memory CD8 T cells as demonstrated by simultaneous staining of CD8 T cells with an MHC tetramer that presents the LCMV epitope and a second MHC tetramer that presents the cross-reactive VV epitope. These results demonstrate that private specificities of preexisting memory CD8 T cell responses control the pattern of cross-reactivity upon secondary infection with unrelated viruses.

Features and consequences of heterologous immunity

Heterologous immunity differs from classic homologous immunity in several key aspects (Table I). As demonstrated in mouse studies, heterologous immunity can have beneficial and harmful effects (Table II). It has, for example, been shown to confer partial protection against viral infections that are otherwise lethal (4), but can also be associated with more severe immunopathology, especially in the absence of a rapid, neutralizing antibody response (1, 4). Furthermore, the absence of reciprocal protection is remarkable. For example, whereas LCMV-immune mice display protective immunity upon VV challenge, the reverse sequence of infections does not confer protective immunity upon LCMV challenge (4).

In the human study by Urbani et al., both patients with cross-reactive T cells developed persistent HCV infection despite vigorous HCV-specific T cell responses and severe, presumably immune-mediated, liver injury (5). Since spontaneous recovery from and protective immunity against HCV infection (6, 15–18) are commonly associated with vigorous HCV-specific T cell responses, why was the vigorous T cell response of these two patients unable to clear the infection? A similar example for viral persistence despite vigorous, cross-reactive T cell responses has been described recently for dengue virus infection (19). After clearance of primary dengue viral infection, subsequent infection with different dengue serotypes results in hemorrhagic fever without efficient viral control. Although dengue virus serotypes are not completely unrelated, they are sufficiently different in that they do not induce a neutralizing antibody response. But cross-reactive CD8 T cells do develop and have been implicated in causing severe immunopathology without efficient viral control (19).

A key characteristic of heterologous immunity that may account for this inability to clear the secondary infection

Table I. Features of heterologous immunity compared to classic (homologous) immunity

	Heterologous immunity	Classic (homologous) immunity
Definition	Infecting virus unrelated to a previously encountered virus	Infecting virus identical to a previously encountered virus
Clinical course		
Outcome	Clearance or persistence depending on the frequency and nature of cross-reactive T cells	Clearance
Immunopathology	Can be severe	Attenuated compared to primary infection with the same virus
Antibodies		
Neutralizing antibodies	Absent	Often present
CD8 T cells		
Kinetics	Early lymphopenia due to cytokine-induced apoptosis	Less lymphopenia due to effective control of the challenge virus
Specificity	Crossreactive, but not necessarily reciprocal	Same epitopes
Hierarchy	Altered, as crossreactivity is often directed towards subdominant epitopes	Maintained from primary infection
Breadth	Narrow, few epitopes	Broad, many epitopes
TCR repertoire	Restricted	Diverse
TCR affinity	Can be low	High
CD4 T cells	May confer crossreactivity, but its implications are not yet defined	Essential part of immunity

Table II. Examples of beneficial and harmful effects of heterologous immunity

	First virus	Second virus	Outcome	Reference
Beneficial effects				
Mouse studies				
	LCMV	Pichinde virus	Reduced Pichinde virus titer	(1)
	LCMV	VV	Reduced VV titer, increased survival upon otherwise lethal VV dose	(1)
	Pichinde virus	VV	Reduced VV titer	(1)
	Murine cytomegalovirus	LCMV	Reduced LCMV titer	(1)
	Murine cytomegalovirus	VV	Reduced VV titer	(1)
	Influenza virus	VV	Reduced VV titer	(3)
	Influenza virus	RSV	Protection against weight loss, illness and lung eosinophilia in G-primed RSV-infected mice	(28)
Harmful effects				
Mouse studies				
	LCMV	VV (intraperitoneal infection)	Acute fatty necrosis	
	LCMV	VV (intranasal infection)	Bronchiolitis obliterans	(2)
	LCMV	RSV	Increased RSV titer	(29)
	Influenza virus	LCMV	Increased LCMV titer, enhanced mononuclear infiltrate	(3)
	Influenza virus	Murine cytomegalovirus	Increased MCMV titer, enhanced mononuclear infiltrate	(3)
Human studies				
	Influenza virus	HCV	Fulminant hepatitis	(5)

RSV, respiratory syncytial virus.

is the altered epitope hierarchy and often narrow focus of the cross-reactive T cell response (Table I). In both patients with fulminant hepatitis C, HCV-specific CD8 T cell responses were narrowly focused on the cross-reactive epitope (5), whereas other patients with less symptomatic or completely asymptomatic clinical presentation displayed a broader immune response (5–7, 9). This deviated hierarchy was likely due to the preferential expansion of cross-reactive memory T cells, which have a low activation threshold and may outpace the priming of naive T cells. Furthermore, cross-reactive memory T cells often display a low affinity TCR (Table I), and low affinity TCR stimulation may elicit different effector functions compared with full TCR stimulation (20, 21). Cytotoxicity, a key function in immunopathology, has been described as requiring only weak TCR signals. Thus, heterologous immunity may result in epitope-specific T cells with an altered effector profile, which together with their narrow focus may cause immunopathology and the inability to clear the secondary virus.

Functionally altered CD4 T cell responses may also contribute to immunopathology and incomplete protection under conditions of heterologous immunity. An important role of CD4 T cells has been indicated in murine studies, in which adoptive transfer of both CD8 and CD4 subsets from LCMV-immune mice into naive mice resulted in heterologous immunity upon subsequent infections with either Pichinde virus or VV (1, 2). However, the function of heterologous CD4 T cell immunity has not been characterized so far and may clarify this point. Alternatively, it is also possible that cross-reactive CD4 T cells are completely absent and that the rapid expansion of cross-reactive memory CD8 T cells may outpace the induction of new, primary CD4 T cells. This may be especially detrimental for the outcome of HCV infection, because CD4 T cell responses are associated with HCV clearance (22), loss of CD4 T cell responses results in HCV recurrence (23), and in vivo depletion of CD4 T cells from HCV-immune chimpanzees abrogates protection upon subsequent HCV rechallenge (24).

Conclusion

The discovery and further analysis of heterologous immunity has simultaneously simplified and complicated our understanding of infectious diseases. As described in mouse models of virus infection, cross-reactivity and heterologous immunity are an intrinsic part of T cell biology and not incidental events (4). These studies have provided clinical investigators with an immunological explanation for the observation that memory T cells against specific viral epitopes are often observed in healthy subjects who lack any evidence of current or past infection with that virus (8). They have also provided a possible explanation for the development of extreme immunopathology in some viral infections, as described in this issue (5).

In the future, studies on cross-reactivity and heterologous immunity may be important to understand why infections with certain viruses such as measles, mumps, varizella zoster, and Epstein-Barr virus run a more severe course if acquired during late adulthood than during early childhood and why certain infections appear to predispose to the development of autoimmune diseases (25) or to the rejection of transplanted grafts (26). Many more cross-reactive T cell responses may remain to be discovered, including those that are based on conformation rather than on sequence similarity between peptide epitopes (27). This task has suddenly become much more difficult. As the paper by Kim et al. (14) demonstrates, both clinical and basic immunologists are now faced with the difficulty of interpreting individualized immune responses. Private specificities of memory T cells exist not only in patients but also in inbred mice, and heterologous immunity is determined by the unique private specificities of these memory T cell populations.

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