Review Article

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MHC immunoevasins: protecting the pathogen reservoir in infection

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Abstract: Alteration of antigen recognition by T cells as result of insufficient major histocompatibility complex (MHC)-dependent antigen-presenting function has been observed in many cases of infections, particularly in *in vitro* systems. To hide themselves from an efficient immune response, pathogens may act on MHC-related functions at three levels: (i) by limiting the number of potential antigens that can be presented to naïve T cells; (ii) by synthesizing proteins which directly affect MHC cell-surface expression; and (iii) by altering the normal intracellular pathway of peptide loading on MHC. Here, we review examples of pathogens' action on each single step of MHC function and we suggest that the result of these often synergistic actions is both a limitation of the priming of naïve T cells and, more importantly, a protection of the pathogen's reservoir from the attack of primed T cells. The above mechanisms may also generate a skewing effect on immune effector mechanisms, which helps preserving the reservoir of infection from sterilization by the immune system.

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In medical practice, it is frequently observed that an infectious disease, effectively controlled at clinical level, is not fully controlled at microbiological level because the infectious agent cannot be eradicated by the combined action of therapeutic treatment and immune response. The potential of some microbes to persist endlessly in the host is a dramatic evidence of the accomplishment of strategies to elude the immune response. Apparently, no particular phase or arm of the vertebrate immune response is entirely safe from pathogen-evasion strategies. Indeed, microbes exhibit an extremely sophisticated biotechnological skill in the interactions with the host (1). In particular, *in vitro* analysis of host–pathogen interactions has revealed a plethora of alterations affecting both the expression and the function of major histocompatibility complex (MHC) molecules, the cell-surface receptors which bind and present antigenic peptides to T lymphocytes. MHC class I molecules, expressed on virtually all nucleated cells, bind peptides from endogenously synthesized

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proteins and present them to $CD8^+$ T cells (cytolytic T lymphocytes or CTL); MHC class II molecules, expressed on few cell types, defined antigen-presenting cells (APC), bind peptides derived from internalized exogenous proteins, and present them to $CD4^+$ T-helper (Th) cells. Triggering of Th cells is necessary to initiate the immune effector mechanisms leading to both cellular (CTL) and humoral (antibody) responses (2).

Pathogens and MHC

Many viruses possess genes whose products seriously impair MHC-I expression in infected cells. Remarkably, impaired MHC-I expression and/or function may be related, in a Mendelean fashion, to the action of the product of single viral open-reading frames (ORFs) (3). Following the terminology introduced by Reddehase (4), we will refer to these genes or gene products as MHC-I immunoevasins. Bacteria, instead, with few exceptions (5) do not usually impair MHC function by downregulating MHC-I expression. In some cases, bacterial infection may actually result in MHC-I upregulation. For example, we have observed an increase in surface MHC-I molecules in macrophage phagocytosing a broad range of bacteria (6; A. De Lerma Barbaro, unpublished data). This overexpression is not related to a direct effect of the infectious particle or to soluble substances (toxins) of microbial origin; instead, it is the result of autocrine factors produced by the cell itself.

In sharp contrast to MHC-I, MHC-II pathway can be altered by a wide array of pathogens including viruses, intracellular, and extracellular bacteria as well as protozoa and multicellular parasites (3–14). Given the role of MHC-II molecules in triggering the Th cells, impairment of MHC-II function would be a major goal for most pathogens as it would block all the effector arms of the immune system. With few exceptions (10–15), little is known on pathogen's gene products involved in MHC-II dysregulation, and the identity of most MHC-II immunoevasins remains elusive.

Infectious agents may assail the integrity of MHC function by different routes. This is related to the fact that the MHC/peptide complexes are involved at various levels and at different time points during the generation of an acquired immune response. They are involved in the priming of naïve T cells, in the identification of the pathogen reservoir, and in the regulation of immune response (Fig. 1).

Priming of naïve T cells and identification of the reservoir of infection pertain to both MHC-I- and MHC-II-related functions. The 'regulatory' function of MHC during immune response, still rather ill defined, involves mostly the MHC-II molecules.

Pathogens' strategies to limit and/or elude priming of naïve T cells

It is intriguing that primary T-cell responses against pathogens are often directed against a small number of pathogens' gene products (16). A clear picture of the cellular and molecular events leading to the above phenomenon is still incomplete. Nevertheless, a series of findings point to the important role of dendritic cells (DCs) and their MHC molecules.

DCs are the main APCs for priming naïve T cells. DC engulf the antigen in the periphery, process it, and become functionally mature while migrating to secondary lymphoid organs where they meet and stimulate T cells (17). It has been found that some viruses can provoke a functional paralysis of DC maturation (18). Furthermore, certain viruses encode ORFs whose products directly affect the MHC-I expression pathway. It is likely that the above events can produce a restriction in the number of pathogen's products effectively available for priming (19).

Herpesviruses are an attractive model system to elucidate the hypothesis of impaired priming of naïve T cells by means of MHC-I deregulation. The repertoire of epitopes recognized by MHC-I-restricted CTLs derived from proteins of HSV, a γ-herpesvirus, HCMV, an α-herpesvirus, EBV, a β-herpesvirus, and the influenza A virus, are presented in Table 1. Although the HSV-2 genome encode about 85 polypeptides (20), CTL responses seem to be focused on only few proteins. In particular, $CD8^+$ T-cell clones infiltrating genital HSV-2 lesions (21) consistently recognize only one immediate early (IE) gene product, ICP0, and two late (L) gene products (UL47 and UL49). Likewise, in C57BL/6 mice, infection with HSV-1 elicits a CTL response that is directed almost entirely to an immunodominant epitope of gB ($gB_{498-505}$) (22). Human CMV is a virus that encodes more than 200 polypeptides (20). A series of studies have identified only a small number of CMV antigens recognized by CTLs. These include the virion protein pp65 and the major IE protein IE-1 (4). Although recent analyses conducted by sophisticated detection systems (23, 24) revealed that the spectrum of epitopes from HCMV proteins capable to be recognized *in vivo* by $CD8^+$ CTL is broader than previously thought, still the number of HCMV proteins effectively recognizable is very restricted compared to the 200 proteins made by the virus. In particular, an analysis conducted by a combined algorithm-based searching for MHC-I-binding epitopes and *ex vivo* assessment of $CD8^+$ T cells in healthy seropositive individuals showed the existence of memory T cells for eight distinct proteins of HCMV. Interestingly, the hierarchy and immunodominance of viral protein-specific CTLs was again in favor of epitopes in the previously recognized pp65 and IE-1 viral proteins (23). As a matter of fact, both HSV and CMV herpesviruses productively infect DCs, causing in these cells an impairment in

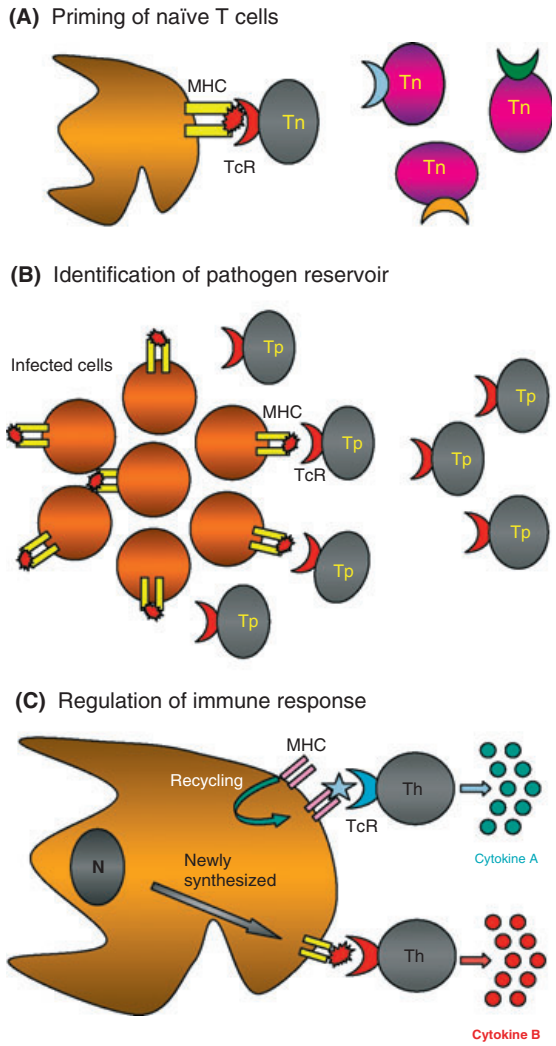

Fig. 1. **Major histocompatibility complex (MHC) drives acquired immune response acting on three phases.** MHC class I (MHC-I) molecules are expressed on virtually all cells. MHC class II (MHC-II) molecules are constitutively expressed on few cell types such as dendritic cells (DCs), B cells, and macrophages; however, their expression can be induced in many cell types, particularly during inflammation generated by pathogen infection. Here, MHC-I and MHC-II molecules are depicted with a unique symbol. MHC molecules act on three levels of the immune response against pathogens. (A) They bind antigenic peptides (✸) derived from pathogens' proteins and present them to virgin naïve T cells (Tn) (CD4 and CD8) for priming. The priming function is mainly carried out by DCs, which are considered the prototype of professional antigen-presenting cells. (B) MHC molecules expressed on infected cells present pathogen-derived peptides to primed T cells (Tp) and help the immune effector cells to attack and eliminate the pathogen's reservoir of infection. (C) MHC molecules, particularly MHC-II molecules, may regulate the immune response by acting both on the priming and on the functional maturation of polarized forms of T-helper (Th) cells, as Th-1 and Th-2 cells. This may be achieved by the alternative or preferential usage of recycling *vs* newly synthesized MHC-II molecules which are loaded with distinct antigenic peptides (✸, ★). T-cell recognition of the distinct MHC-II-peptide complexes can generate a different pattern of effector cell response depicted in the figure as the secretion of alternative cytokines. TcR, T-cell receptor.

antigen-presentation capacity (25, 26), and both herpesvirus encode the most efficient inhibitors of MHC-I pathway described so far (3).

On the contrary, the $CD8^+$ CTL repertoire against the proteoma of both influenza A virus and EBV is broad. During human flu infections, it is possible to find CTL responses directed against six of the 10 proteins encoded by the virus (27). In mice, a wide-ranging analysis of antigenic peptides from influenza A PR8 strain proteome has shown CTL responses directed against 10 of the 11 viral products (28). Similar findings have been described for EBV infections: among six viral products analyzed, two IE antigens and three E antigens elicit $CD8^+$ T responses (29). These two viruses do not interfere directly with the MHC-I pathway, although the EBNA from EBV impairs its own proteasome-mediated degradation preventing formation of peptides that may be bound by MHC-I molecules (30). Importantly, both influenza virus and EBV do not inhibit functional maturation of DC (31, 32).

The correlations summarized in Table 1 offer the hint for an additional consideration related to the availability and immunogenicity of a viral antigen. A narrowed CTL response against pathogen's products can be observed for viruses that bear MHC-I immunoevasins. ICP47 from HSV and the products of the US2-US11 gene region of HCMV strongly downregulate MHC-I cell-surface expression (3). Thus, potentially immunogenic peptides from proteins that during the life cycle of the two viruses are synthesized downstream of the ICP47 and US2-US11 immunoevasins, respectively, may have an objective difficulty in being presented by MHC-I molecules of the infected DCs. The host, however, can still counteract the effect of immunoevasins in several ways: the APCs that can be infected may be diverse and thus display a different susceptibility to the action of immunoevasins (23, 24, 33); the action of immunoevasins can be overcome by the capacity of uninfected APCs to cross-present viral antigens that cannot be presented by infected cells (34); lastly, immunoevasins may have different inhibitory potential on MHC-I function and/or expression depending upon the MHC-I allele present in the infected host (35).

Identification of the cellular reservoir of infection

It has been argued that the goal of intracellular pathogens is not to kill the cell host, but to replicate and spread to an extent compatible with survival of both pathogen and host. Therefore, the maintenance of a cellular reservoir (Fig. 1B) can be considered a priority for pathogens. Experimental evidence documents in many cases disruption of the MHC-II pathway by intracellular pathogens in the

Correlation between viral infection of dendritic cells (DCs), their antigen-presenting cell (APC) function, and MHC-I-restricted response of immune cytolytic T lymphocytes (CTL) effector cells

Virus	Genes that affect MHC-I pathway	Interaction with DCs	$CD8^+$ T-cell epitopes identified
Herpes simplex virus	ICP-47 (3, 36)	Infection of DC causes inhibition of T-cell-stimulatory capacity (25)	Human: three IE and four L products (21) Mouse: mainly focused to the gB L product (22) Viral genome encodes 85 proteins
Cytomegalovirus	US2, US3, US6, US11, US18, m04, m06, m152 (4, 36)	Infection of DC causes downregulation of MHC molecules and reduced antigen-presentation capacity (26)	Human: dominant for one L gene encoding *pp65* and for one IE gene encoding *UL123* (4, 19); detectable for L gene products pp50, p150, pp28, gB (23) Mouse: one IE and six E/L products (4) Viral genome encodes >200 proteins
Epstein–Barr virus	EBNA-1 (30)	Infection induces apoptosis in precursor monocytes but not in DCs (32)	Two IE and three E gene products among eight viral proteins tested (29) Viral genome encodes >80 lytic cycle products
Influenza A virus	Unknown	Infection causes DC maturation and release of IL-12 (31)	Human: six viral products (27) Viral genome encodes only 10 proteins Mouse: 10 viral products (28) Viral genome encodes only eleven proteins

Table 1

reservoir of infection (3–5, 7, 8, 36). Thus, if the infected cells can function as surrogate APCs, an inhibitory action on the MHC-II pathway could be particularly useful to intracellular bacteria and protozoa (whose antigens are presented mainly by MHC-II) for overcoming sterilization of the reservoir mediated by primed Th cells (Fig. 2). In fact, these cells need recognition of MHC-II–peptide complex to be stimulated but have less requirements than naïve T cells for accessory costimuli (14, 37). Within this frame, it should be stressed that pathogen-mediated MHC-II downregulation in APCs may affect a major effector function of Th-1 cells, the production of interferon-gamma (IFN-γ), whose microbicidal activity within the reservoir of infection has been firmly established (38).

In the course of viral infections, analogous circumstances could also take place. Presentation of non-viable virions via MHC-II and its relevance to the help of B lymphocytes and CTL have been well documented (14). Moreover, viral antigens can be loaded on MHC-II also in productively infected cells; in fact, viral structural proteins move to the endosomal compartment, presumably during the budding of infective particles. In this compartment, viral peptides can be bound by MHC-II molecules, either newly synthesized or recycling from the cell surface (8, 39), and be expressed on the cell surface where they can be recognized by $CD4^+$ T cells. These T cells can mediate a direct sterilization of the reservoir of infection through an intrinsic cytotoxic function (40), or through secretion of cytokines that enhance cellular antiviral function. For instance, IFN-γ induces expression of 2′,5′-oligoadenlyate synthetase that mediates degradation of viral RNA (41). Therefore, viruses could improve their fitness by interfering with MHC-II-presentation pathways in productively infected cells as well.

The strategy for improving the fitness of a pathogen for the host can be exerted also in another way. In macrophages undergoing bacterial phagocytosis, the reservoir of infection is an heterogeneous population in which single cells could contain very different pathogen loads, and we have observed an inverse correlation between MHC-II expression and bacterial burden *in vitro* (A. De Lerma Barbaro, unpublished data). From the pathogen's side, to achieve an enhanced fitness and to establish a hidden reservoir protected from immune attack, it could be sufficient to escape Th-mediated sterilization in only few cells among those infected (Fig. 2). Studies devoted to the analysis of MHC-II downmodulation in biopsy specimens of infected tissues, such as those reported for CMV-infected lung tissues (42), could be extremely useful in clarifying the importance of the differential pathogen load for the establishment of a persistent infection. Within this frame, the additional inhibitory effect on MHC-II exerted not only on the infected cells but also on bystander APCs by certain viruses that induce the production, or code for a homolog, of the MHC-II-inhibitory cytokine IL-10 (43) should be also considered.

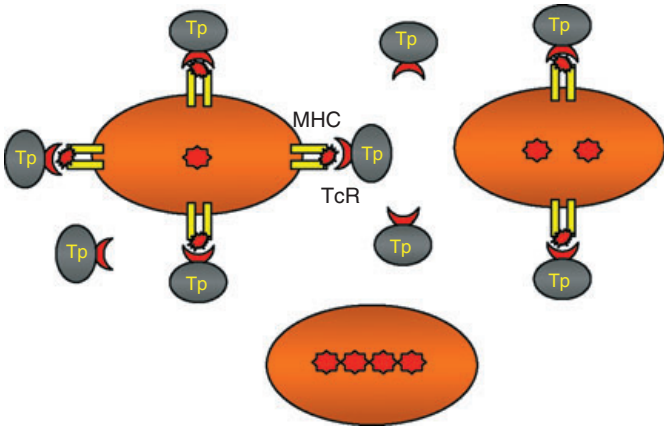

Fig. 2. **Inverse correlation between pathogen load and major histocompatibility complex (MHC) expression.** Downregulation of MHC expression induced by pathogens (✸) has been shown in many cases of viral and bacterial infections. At the single cell level, the intensity of the MHC downregulation correlates with the intracellular pathogen load. This, in turn, can generate a differential response of primed immune effector T cells (Tp) against the infected cells, resulting in the preferential elimination of those cells with a low pathogen load. Thus, the persistence of infection despite an active and performant immune response, may increase the fitness of the pathogen and generate, by a vicious circle, a skewing of the immune response toward a non-protective immunity. TcR, T cell receptor.

The assertions made for MHC-II may likely apply to MHC-I as well. Indeed, viruses would increase their fitness if they can protect the reservoir of infection from sterilization by MHC-I-restricted CTL effectors, as it has been shown during Kaposi herpes virus (KSHV) infection (44). If from one side, MHC-I downmodulation would protect infected cells from CTL recognition, from the other side these cells may become potential target of natural killer (NK)-mediated lysis. It is of relevance that also against NK attack, the KSHV virus has developed counteracting mechanisms by synthesizing the K5 protein which inhibit both MHC-I expression and NK-mediated cytotoxicity (45). Some viral pathogens have developed strategies for escaping MHC-mediated recognition and creating a hidden reservoir that rely on limiting the number of potential antigenic peptides recognizable by effector CTLs. For example, the occurrence of frequent spontaneous genomic mutations during infections by lentiviruses, such as HIV and SIV (46, 47), may generate mutant peptides that no longer bind, or bind with reduced affinity, to MHC-I molecules (48); alternatively, the mutation can affect the processing of mutant protein leading to an impaired trimming of the peptide (49).

Regulatory function of MHC/peptide complex

It is becoming apparent that the MHC–antigen peptide complex plays an important role not only on the efficacy but also on the quality of T-cell stimulation. Some data support the view that peptide loading onto recycling *vs* newly synthesized MHC-II molecules may produce conformationally distinct complexes distinguishable by TcRs (50, 51) (Fig. 1C). Epitopes loaded on recycling MHC-II are often located on structurally simple, non-globular domains of the protein (52, 53). For example, immunization with reduced and alkylated MSP-1 from malaria merozoide allows C-terminal region, usually loaded on newly synthesized MHC-II, to be presented by recycling MHC-II (52). More recent data suggest that mild acidification and disulfide bond reduction of influenza hemagglutinin, in the absence of proteolysis, provides sufficient processing for loading on recycling MHC-II (54), confirming previous observations that an entire protein subunit may indeed be loaded on recycling MHC-II (55). Moreover, it has been shown that VacA from *Helicobacter pylori* specifically inhibits the Ii-dependent pathway of antigen presentation on newly synthesized MHC-II molecules, leaving unaffected the Ii-independent pathway on recycling MHC-II (10). Bm-CPI-2, a cysteine protease inhibitor from *Brugia malayi*, displays remarkable similarities with VacA as a suppressor of MHC-II presentation (11). Taken together, all the above observations strongly suggest that redirection of peptide loading on recycling MHC-II molecules may substantially change the repertoire of peptide antigens which can be presented to T cells. At the same time, this event can generate a qualitatively different hierarchy in the T-cell clones involved in the immune effector function. One possible skewing effect can be a biased Th-1/Th-2 polarization (56). The selective advantage of the pathogen would consist in redirecting the course of acquired immunity toward a non-protective response rather than in avoiding recognition. A plausible link of these speculations to the clinics would be the observation that the immunopathological sequelae of *H. pylori* infection may consist in an autoimmune syndrome characterized by a Th-1 profile (57).

Conclusions and future perspectives

Interference of infectious agents on the MHC-I and MHC-II pathways may play a relevant role *in vivo* not only in preventing T-cell priming but, more importantly, in hiding and protecting the cellular reservoirs of infection from sterilization. Future studies focused on the connection between molecular aspects (i.e., ORFs that impair MHC function), and cellular events (the interaction between the pathogen and the APC, as well as the T-cell repertoire effectively stimulated) will be instrumental to better clarify host–pathogen interaction during infectious diseases.

Moreover, it will be of paramount importance to elucidate the role of 'ectopic' expression of MHC-II *in vivo* in non-professional APCs

that can be reservoir of intracellular pathogens. Within this frame, more emphasis should be given to the identification of MHC-II immunoevasins. HCMV encodes the three major viral MHC-II immunoevasins identified to date, namely U2, U11, and U3, and it is interesting to note that these molecules affect MHC-I pathway as well. Unfortunately, since a suitable animal model for HCMV infection is lacking, it is not possible to study the *in vivo* relevance of these genes by deletion analysis (58). Microbes bearing a transgenic GFP (59) can be used to identify infectious particles in tissues, the relative pathogen load in cells of the affected tissue, and the corresponding expression of MHC-II molecules. The targeted inactivation of the promoter IV of the *AIR-1* gene encoding the MHC-II transactivator (CIITA) (60, 61) produces mice whose putative APCs of non-hematopietic origin cannot be induced by IFN-γ for MHC-II expression (62). These mice could be a valuable *in vivo* model to study the relevance of MHC-II inducibility in the clinical course of infections by some intracellular pathogens such as chlamydia, salmonella, and CMV that colonize cellular reservoirs distinct from macrophages.

These studies will increase the knowledge of the pathogen–host interactions and will contribute to the design of novel strategies for vaccine production.

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