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Immunity to Polyomavirus Infection: The Polyoma Virus-Mouse Model

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Abstract

A ubiquitous clinically silent murine pathogen, polyoma virus has enjoyed long-term co-evolution with the mouse, a highly tractable and genetically and immunologically informative small animal model. Thus, polyoma virus has provided a valuable experimental construct to decipher the host immune mechanisms that come into play to control systemic low-level persistent viral infections. Impaired immunosurveillance for infected cells puts the murine host at risk both to injury resulting from excessive direct virus cytolysis and development of virus-induced tumors. In this review, we present our current understanding of the multifaceted immune response invoked by the mouse to maintain détente with this potentially deleterious persistent natural pathogen, and discuss implications of these studies for therapeutic interventions for human polyomavirus infection.

Keywords

Polyoma virus; Mouse; Cellular Immunity; Humoral Immunity

1. Introduction

The discovery of mouse polyoma virus (MPyV) over fifty years ago may be credited to the prevailing concept at the time that the immune system operates primarily to protect the host against neoplasia. Working along parallel lines, Gross' group and that of Stewart and Eddy independently sought to isolate a leukemogenic viral agent by injecting cell-free extracts from leukemic AKR mice into newborn mice [1,2]. The use of newborn mice was prompted by Medawar's seminal studies indicating a predisposition of the newborn immune system to immunological tolerance. By extension, an ontologically "immature" immune system would be at a disadvantage in mounting anti-tumor immunity and be prone to viral leukemogenesis. The unexpected appearance of salivary gland tumors in these neonatally inoculated mice was the first telltale sign of the polyoma viral "contaminant". The concomitant leukemia and its associated perturbations on host immunity likely also abetted the development of MPyV-induced tumors. Thus, the serendipitous discovery of MPyV, the founding member of the

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polyomavirus family, may be viewed as an early experimental demonstration that immune surveillance contributes to host defense against neoplastic disease.

MPyV is a ubiquitous silent persistent pathogen in wild mice. Natural transmission of the virus most likely occurs through a respiratory route. MPyV is shed from infected carriers in the urine and is also found in saliva and feces. The virus is stable and recoverable from bedding and nesting materials, and from aerosols as well. Yet, we lack a mechanistic understanding for how these lytic viruses maintain life-long persistent infections in their natural host reservoirs. Unlike herpesviruses, there is no evidence that polyomaviruses establish a latent state of infection or that their genomes can be excised after integration into host genomic DNA; moreover, integrated viral genomes typically harbor deletions in coding and noncoding regions that cripple viral viability [3]. The term “reactivation” is often wielded in the literature to designate low-to-high shifts in polyomaviral replication levels in immunocompromised hosts, terminology that infers a pre-existing latent state. Polyomaviruses most likely persist as infectious virus in semi-permissive cells, where a low virion output is minimally injurious to the host; changes in the microenvironment (e.g., inflammatory mediators, tissue repair) convert these or neighboring cells into a state fully permissive for productive viral infection. Support for this possibility comes from evidence that mesothelial cells can maintain SV40 episomally and continuously shed low-output infectious virus *in vitro* [4], and that renal injury in adult mice months after MPyV inoculation results in high-level productive viral infection [5]. Not unexpectedly, organs harboring high levels of viral DNA preferentially develop MPyV-induced tumors [6]. Taken together, these studies argue that development of tumors may be deemed an overt manifestation of the failure of immunologic control of persistent MPyV infection. Thus, the host must muster and maintain a multi-pronged (i.e., cellular and humoral) immune response to contain this “smoldering” viral infection.

Because polyomaviruses have a narrow host range that restricts productive infection to their natural hosts, the only tractable system for studying polyomavirus pathogenesis and immunity is the MPyV infection model. Given the ease of MPyV genome manipulation and propagation of the virus in tissue culture, virologic determinants for MPyV replication, cellular transformation, and tumor induction have been and continue to be uncovered [7]. The “plasmid-like” ~5-kb double-stranded covalently closed MPyV genome readily lends itself to mutagenesis, which has permitted fine dissection of the viral determinants for MPyV pathogenesis both at the level of interaction with host cells as well as systemically at the level of the host’s antiviral immune response. While the deleterious effects of high-level chronic viral infections (e.g., HIV, HCV in humans; LCMV clone 13 in mice) on the host’s antiviral immunity are well documented [8], we know surprisingly little how healthy individuals maintain long-term control of viruses that establish low-level persistent infection. MPyV offers an attractive model for deciphering the mechanisms of immunological control of this large class of persistent viral infections. In addition, MPyV provides a model for probing the pathogenesis of morbidity associated with human polyomavirus infection in immunocompromised individuals, such as BKV-associated nephropathy in kidney transplant patients. Indeed, the spectrum of MPyV-induced diseases in immunodeficient mice reveals the important roles different components of the innate and adaptive immune system play in controlling MPyV infection (Table 1). In this review, we will discuss our current understanding of immunity to polyomavirus infection drawn from studies in the MPyV infection model.

2. T cells mediate surveillance for MPyV-induced tumors

The importance of immune surveillance for MPyV-induced tumors has long been appreciated. Early studies using neonatally thymectomized mice, congenically athymic mice, and adoptive transfer of splenocytes from MPyV-infected mice demonstrated that T cells prevent tumors induced by MPyV infection [9–11]. Later studies showed that CD8 T cells are the primary

immunocyte population required for protection against MPyV-induced tumors, as evidenced by the inability of mice subjected to antibody-mediated CD8 T cell depletion to reject a syngeneic tumor cell challenge, the ability of a synthetic class I MHC-binding viral peptide to confer protection against a polyoma tumor cell challenge, and the increased tumor susceptibility of mice incapable of MHC class I-restricted antigen presentation (i.e., $\beta_2m^{-/-}$ mice) [12–14]. In this connection, it is interesting to note that $CD8^{-/-}$ mice are MPyV tumor-resistant [14,15], a surprising phenotype that may be explained by the capacity of these mice to mount an MHC class I-restricted $CD4^{-/-}CD8^{-/-}$ anti-MPyV T cell response [16]. Humoral immunity does not appear to contribute substantially to polyoma tumor resistance, given that both tumor-resistant and tumor-susceptible mice generate strong neutralizing anti-polyoma antibody responses, administration of hyperimmune serum post-inoculation fails to protect against polyoma-induced tumors, and μ MT knockout mice (i.e., no mature B cells) retain tumor resistance [17–20].

3. Host determinants affecting MPyV immunosurveillance

Inbred strains of mice vary in their susceptibility to MPyV tumorigenesis when inoculated as neonates [21]. The application of near-lethal doses of whole-body irradiation has been used to distinguish mouse strains having immunological vs. nonimmunological forms of resistance, with the latter strains having a profound blockade to virus replication and/or dissemination by a presently unknown mechanism [22,23]. Among the former mouse strains, two germline-encoded loci stand out as autosomal dominant co-determinants conferring high susceptibility to MPyV-induced tumors: (1) the MHC haplotype H-2^k [21]; and (2) the endogenous superantigen (SAG) encoded by the mouse mammary tumor provirus *Mtv-7* [23]. SAGs crosslink cell-surface MHC class II proteins to the variable regions of the β -chain ($V\beta$) of the antigen-binding component of T cell receptor (TCR) heterodimers; different SAGs discriminate among TCRs containing each of the ~20 $V\beta$ chain families. When introduced exogenously (e.g., bacterial enterotoxins) SAGs drive large-scale T cell activation, but, as endogenous host proteins, SAGs mediate negative selection of thymocytes according to their $V\beta$ binding specificity. The *Mtv-7* SAG culls the peripheral T cell repertoire of T cells expressing TCRs using $V\beta 6$ and $V\beta 8.1$, which fortuitously are expressed by most MPyV-specific CD8 T cells in H-2^k mice [23,24]. Thus, among H-2^k mice, those carrying *Mtv-7* are at a sizeable numerical disadvantage for anti-MPyV CD8 T cells to *Mtv-7*-negative mice, resulting in higher and longer duration MPyV replication in the former animals.

In addition to this quantitative disparity, autochthonous receptors come into play to diminish the functional integrity of the antiviral CD8 T cells in MPyV tumor-prone mice. The residual anti-MPyV CD8 T cells in tumor-susceptible H-2^k *Mtv-7*⁺ mice are fully differentiated effectors, but their cytotoxic effector function is restrained by CD94/NKG2A, a heterodimeric inhibitory receptor expressed by both murine and human NK cells and a subset of CD8 T cells [25]. CD94/NKG2A's ligand is the nonclassical MHC class I (class Ib) molecule Qa-1^b (HLA-E is the human ortholog) complexed to an oligopeptide derived from the leader sequence of classical MHC class I (class Ia) molecules. MPyV-specific CD8 T cells in tumor-susceptible mice prematurely upregulate CD94/NKG2A receptors, causing termination of cytotoxic activity prior to clearance of infectious virus [25]. In H-2^k *Mtv-7*⁺ mice viral infection more effectively outcompetes the smaller antiviral CD8 T cell response, resulting in a higher setpoint of viral persistence and more disseminated viral burden than in resistant mice [26]. In this setting, negating the cytotoxic function of anti-MPyV CD8 T cells may represent a host protective mechanism against immunopathology. Recent studies have revealed that a panoply of inhibitory receptors in addition to CD94/NKG2A are expressed by virus-specific CD8 T cells in chronically infected hosts (e.g., PD-1, LAG-3, Tim-3), with the number of co-expressed inhibitory receptors directly related to the severity of the infection [27]. Whether these inhibitory receptors are expressed in a hierarchal fashion, preferentially operate in different

tissues, or are selectivity engaged by different persistent viral infections, is not known. Importantly, recent experimental evidence that antiviral CD8 T cell function can be restored *in vivo* by antibody-mediated blockade of inhibitory receptors or their ligands has invigorated efforts to target these receptors as a novel therapeutic intervention for chronic viral infections in humans [27,28].

While the Mtv-7 SAG perturbs adaptive antiviral immunity to MPyV infection, host differences in innate immune responses to MPyV infection may also contribute to tumor susceptibility. Mice of an H-2^k wild-derived inbred strain lacking endogenous Mtv proviruses were found to be highly susceptible to MPyV tumorigenesis, and to transmit this susceptibility in an autosomal dominant fashion, yet still be able to generate MPyV-specific CD8 T cells [29]. In contrast to resistant H-2^k mice, however, the cytotoxic function of anti-MPyV CD8 T cells in these susceptible mice rapidly decayed over time post-infection and, rather than producing IFN- γ , these CD8 T cells secreted the immunosuppressive cytokine IL-10 [30]. Immune deviation toward this type 2 cytokine profile appears to be fated at the level of antigen-presenting cells (APCs), which in these Mtv^{null} tumor-susceptible mice preferentially produce IL-10 rather than IL-12 after exposure to infectious virus or MPyV-VP1 virus-like particles (VLPs); B cells (which are nonpermissive for MPyV replication) appear to be a major source of IL-10 [30]. Interestingly, the nonstructural M2 protein of a mouse γ -herpesvirus triggers IL-10 production by B cells, presumably to dampen adaptive antiviral immune responses [31,32]. Additionally, high IL-10 levels are induced by chronic LCMV infection, with IL-10 antibody administration promoting viral clearance [32]. Administration of IL-12 to these Mtv^{null} mice at the time of neonatal inoculation reduced the incidence and increased the latency of tumors, further supporting the concept that early steps in MPyV infection of APCs regulates the differentiation pathway of adaptive anti-MPyV immunity [30].

4. CD8 T cell responses to MPyV infection

Anti-MPyV CD8 T cell epitopes have been defined for inbred mouse strains of the H-2^k and H-2^b haplotypes and the fate and function of epitope-specific CD8 T cells monitored during acute and persistent phases of infection [24,33]. Each of these MHC class Ia (i.e., H-2D and H-2K)-restricted CD8 T cells are directed toward peptides derived from the nonstructural T antigen. In general, the anti-MPyV CD8 T cell response peaks around one week after virus inoculation, then undergoes a 6-fold contraction, with a small population that is stably maintained throughout persistent infection [33,34]. In H-2^b mice, epitope-specific CD8 T cell responses exhibit non-coordinate regulation in their expansion and contraction during acute infection, expression of activation and differentiation cell surface markers, and cytokine effector profiles [33]. Many factors may contribute to this variability in kinetics, magnitude, and function of CD8 T cells directed to different viral epitopes, including competition for infected cells, efficiency in epitope processing and presentation by MHC class I molecules, and the number of precursor naïve T cells of a given specificity in the host's T cell repertoire [35]. While control of acute MPyV infection parallels the expansion of the antiviral CD8 T cell response, it is important to point out that MPyV DNA is detected long-term in the face of functionally competent antiviral CD8 T cells [33,36].

Memory T cells generated in the context of persistent infection, however, are qualitatively distinct from those generated in infections that are completely resolved, and have been referred to as “chronic memory” and “acute memory”, respectively [8,37,38]. Acute memory T cells are maintained by cytokine-driven proliferative renewal, express high IL-7 Receptor- α (CD127) levels, have a lower TCR activation threshold than naïve T cells, and exhibit heterogeneity in the lymph node homing receptors CD62L-selectin and CCR7 [38]. Differential expression in CD62L and CCR7 is commonly used to demarcate memory T cells into central-memory (CD62L^{high}CCR7^{high}) and effector-memory (CD62L^{low}CCR7^{low})

subsets; these memory subpopulations differ in anatomic location, kinetics of effector expression, and homeostatic proliferation [39]. Over time after viral clearance, acute memory T cells gradually acquire a central-memory phenotype, such that this population may rival or even exceed the effector-memory subset; although the relationship between these two acute memory populations has yet to be clarified, the self-renewing, lymph node-resident central-memory T cells may replenish the less proliferative, peripheral tissue-resident effector-memory T cells. MPyV-specific CD8 T cells isolated during the persistent phase of infection are primarily CD127^{int}CD62L^{lo}CD44^{hi}, consistent with an effector-memory phenotype [33]. This pronounced effector-memory bias by chronic memory T cells is also seen in low-level persistent virus infections in humans and in other experimental infection models [37]. Memory CD8 T cell function during chronic infection is profoundly affected by viral load. Chronic infections with high viremia, such as HIV and HCV in humans and LCMV clone 13 in mice, lead to a progressive loss of memory CD8 T cell function, a phenomenon dubbed “exhaustion” [8]. On the other hand, MPyV-specific memory CD8 T cells, like those of most persistent low-level viral infections, generally retain most of their effector functions [40,41]. Perforin mediated killing seems to be particularly important during persistent infection as perforin-deficient mice are associated with higher viral loads and have a reduced ability to kill viral antigen-loaded targets [42].

CD8 T cell function can also be affected by anatomic location. Immune-privileged sites (e.g., eye, testes, and brain) are particularly sensitive to inflammation-associated injury and have mechanisms in place to prevent such pathology. During MPyV infection, the kidney, while not normally considered an immune-privileged organ, contains substantial numbers of antiviral CD8 T cells that are refractory to antigenic peptide-induced IFN- γ and TNF- α production ([43]; P.A. Swanson and A.E. Lukacher, unpublished observations). Although the mechanism(s) for this functional deficit is unknown, one can speculate that direct engagement of inhibitory receptors, immunoregulatory cytokines, or a combination of both could act to inhibit CD8 T cell effector activity in the kidney. If functional impairment is also a characteristic of kidney-resident antiviral CD8 T cells in humans, such “immune privilege” may favor this organ as a repository for human polyomaviruses.

Unlike $\beta_2m^{-/-}$ mice, which lack all MHC class I molecules and are highly susceptible to MPyV-induced tumors, mice lacking only MHC class Ia molecules (e.g., B6.K^{b-/-}D^{b-/-} mice, Table 1) are tumor-resistant and control infection as efficiently as wild type mice [44]. Infected MHC class Ia-deficient mice were found to mount a protective, anti-MPyV CD8 T cell response restricted by the nonpolymorphic MHC class Ib molecule, Q9. A member of the murine Qa-2 family, Q9 is structurally similar to other β_2m -associated MHC class Ia molecules, preferentially binds TAP-dependent nonameric peptides, and is widely expressed [45–47]. Q9-restricted anti-MPyV CD8 T cells recognize a 9mer peptide derived from the overlapping sequence of the minor capsid VP2 and VP3 proteins [44]. Although this is the first MPyV-specific CD8 T cell epitope derived from one of the viral structural proteins, it should be noted that the dominant CD8 T cell response to the human BK and JC polyomaviruses is directed toward a peptide derived from the VP1 capsid protein [48,49]. Because expression of viral structural proteins is typically limited to productively infected cells, the generation and maintenance of CD8 T cells specific for capsid-derived peptides during persistent infection fits with the concept raised earlier that polyomaviruses are maintained in a low-level infectious state. By extension, antiviral CD8 T cells recognizing epitopes from capsid proteins may be needed to keep persistent viral infection in check, and possibly to contain pockets of resurgent viral replication. Additionally, class Ib-restricted CD8 T cells may offer protection from immune selection of epitope-escape viral variants by immunodominant class Ia-restricted antiviral CD8 T cells. From a vaccination standpoint, because Q9 is a nonpolymorphic molecule, peptide immunizations could potentially protect mice from viral challenge across MHC class Ia allogeneic barriers. This discovery of a protective antiviral MHC class Ib-

restricted CD8 T cell response using the MPyV-mouse model should stimulate efforts to uncover class Ib-restricted CD8 T cell epitopes for human viruses. Because MHC class Ib molecules have few polymorphisms, peptide-based CD8 T cell immunization using MHC class Ib-restricted epitopes offers the prospect for far broader coverage than achievable using MHC class Ia-restricted peptides.

5. Effects of acute vs. persistent infection on MPyV-specific CD8 T cell differentiation

Stable numbers of anti-MPyV CD8 T cells are maintained over the course of persistent infection. Yet, when transferred to infection-matched recipients, MPyV-specific CD8 T cells from persistently infected mice fail to proliferate and undergo rapid attrition [50]. This conundrum appears to have been resolved by the finding that virus-specific naïve T cells are recruited during persistent infection [33,50]. Moreover, in contrast to anti-MPyV CD8 T cells primed during acute infection, those generated during the persistent phase of infection are largely CD62L^{high} and CD27^{high}, indicating that these “late-primed” cells share the signature central-memory T cell phenotypic markers [33,50]. Using naïve TCR transgenic CD8 T cells to precisely time when MPyV-specific CD8 T cells are recruited, we have found that the central-memory phenotype becomes more pronounced when priming occurs at later stages of persistent infection (C.D. Pack and A.E. Lukacher, unpublished observations). Priming history, then, may imprint a particular differentiation program on antiviral CD8 T cells that affects their capacity to effectively mediate surveillance for infected cells. Along these lines, virus-associated inflammation has been shown to be detrimental to the quality of the MPyV-specific CD8 T cell response [51]. Conceivably, reduced virus-associated inflammation and/or antigen load during persistent stages of infection may be conducive for generating authentic memory T cells which are capable of self-renewal (e.g., responsive to homeostatic cytokines, such as IL-7 and IL-15) and rapid expansion following re-exposure to cognate viral antigens. Thus, one can envision a conveyor belt scenario where continuous priming of virus-specific naïve T cells is required to resupply the pool of deteriorating antiviral CD8 T cells at early stages of persistent infection, but, as viral load/inflammation diminishes, self-sustaining bona fide memory T cells progressively emerge and assume a larger role in maintaining virus-specific T cell memory.

Costimulation requirements for MPyV-specific CD8 T cell expansion are also affected by the priming environment. Both CD28 and CD40L costimulation are particularly important for MPyV-specific CD8 T cell expansion during the acute phase of infection, as blockade of these pathways acts additively to inhibit the magnitude of this response [52]. However, costimulation requirements shift during the persistent phase of infection as indicated by data showing that combined CD28/CD40L blockade has no effect on memory T cell numbers or *de novo* late priming of MPyV-specific CD8 T cells. Phenotypic analysis of MPyV-specific CD8 T cells in persistently infected mice shows that most of these cells express the costimulatory molecule CD27. In fact, MPyV-specific CD8 T cells primed during persistent infection exhibit a markedly higher CD27 expression level than pre-existing host anti-MPyV CD8 T cells. Expression of this costimulatory molecule during the persistent phase of infection is necessary, as blockade of CD27, in conjunction with CD28 blockade, significantly reduces MPyV-specific CD8 T cell numbers. Thus, antiviral CD8 T cell costimulation requirements during MPyV-infection swing from being CD28- and CD40L-dependent during the acute phase to CD27- and CD28-dependent in persistently infected mice.

6. Contribution of CD4 T cells to MPyV-specific CD8 T cell responses

For acutely resolved viral infections, CD4 T cells are essential for generating long-lasting, functional memory CD8 T cells [53,54]. Although CD4 T cells are dispensable for CD8 T cell

expansion and function during the acute phase of the antiviral response, CD4 T cells are essential for maintenance of memory CD8 T cells, endowing them with the ability to efficiently proliferate and function upon antigen rechallenge [55]. Persistent virus infection may be envisioned as a situation where virus-specific memory T cells are repeatedly driven into recall responses. Consistent with this model, there are far fewer MPyV-specific CD8 T cells in persistently infected CD4-depleted mice compared to wild type mice [56]. However, unlike the CD8 T cell exhaustion seen in CD4-depleted, LCMV clone 13-infected mice, where most of the non-deleted virus-specific CD8 T cells are nonfunctional, the residual MPyV-specific CD8 T cells in CD4-deficient mice are fully functional and capable of responding to rechallenge [8,56].

While priming of naïve MPyV-specific CD8 T cells during the acute phase of infection seems to be largely CD4 T cell independent, de novo generation of MPyV-specific CD8 T cells during the persistent phase of infection does require CD4 T cell help. Studies using bone marrow chimeras revealed that naïve virus-specific CD8 T cells fail to be recruited in persistently infected, CD4-deficient mice [56]. Whether CD4 T cells are required for early events or maintenance of MPyV-specific CD8 T cells primed during persistent infection remains to be elucidated.

7. Role of antibody responses in MPyV pathogenesis

Similar to other virus infections, MPyV induces a neutralizing antibody response. Early reports showed that neonatal mice born to MPyV-immune mothers were resistant to tumor development induced by MPyV inoculation, due to the protective effect of maternal antibodies. Passive immunization with serum from MPyV immune mice also prevented tumor formation of neonatal mice when it was administered before MPyV infection. However antibodies given days after the onset of infection did not protect mice from tumor formation [57]. These and many subsequent observations consistently showed that MPyV infection induces a neutralizing antibody response [58,59], which protects newborn mice from infection during the first week of life when the mice are susceptible to tumor induction by MPyV. In addition to preventing subsequent infection, MPyV-specific antibodies also play an important role in controlling ongoing infection by decreasing the viral load. However B cell responses, alone, are not capable of preventing tumor development [60] (Table 1).

8. Antibody responses to MPyV in immunocompetent mice

Antibodies responses to MPyV are mostly directed against the major capsid protein VP1, rather than the minor capsid components, VP2 and VP3 [61]. Following intraperitoneal MPyV infection of immunocompetent C57BL/6 mice, virus-specific IgM appears in the serum by day 4, at which time levels decrease and serum IgG responses become detectable (~day 7 post-infection), peaking around days 21–28 [62]. Consistent with the serum antibody data, plasma cells secreting virus-specific IgG can be detected by ELISPOT assays in the spleen starting at day 7 and peaking by day 21 [63]. In contrast to other viral infections, such as influenza virus, where natural IgM antibodies are virus specific and neutralizing [64], natural antibodies present in the sera of uninfected mice are not reactive with MPyV [60]. Studies have shown that antiviral B cells, in addition to T cells, contribute to reducing viral load in MPyV-infected immunocompetent mice. B cell-deficient (μ chain KO) mice infected with MPyV as adults had higher virus levels during the persistence phase of infection when compared to infected wild type mice, as determined by PCR [65].

9. Antibody responses in T cell-deficient mice

Studies using MPyV-infected T cell-deficient (TCR $\beta \times \delta$ KO) mice and T and B cell-deficient SCID mice indicate that T cells are not essential for survival during the acute phase of MPyV

infection. However, MPyV-infected SCID mice, which lack adaptive immunity, die within 2–3 weeks after infection from an acute myeloproliferative disease, accompanied by uncontrolled MPyV replication [66]. Remarkably, reconstitution of SCID mice with naïve B cells prior to infection results in complete survival of all animals accompanied by significantly decreased virus levels in all organs tested, indicating that B cell responses generated in the absence of T cells are protective against MPyV. Indeed, sera of MPyV-infected TCR $\beta\gamma$ KO and SCID mice reconstituted with B cells contains MPyV-specific IgM and IgG antibodies, demonstrating the efficient induction of a MPyV-specific T cell-independent (TI) antibody response [60]. MPyV-specific TI IgG antibodies are mostly IgG2a and IgG2b isotypes. Insight into the contribution of MPyV-specific TI antibodies to the overall antiviral antibody response comes from data showing that the MPyV-specific antibody titers in sera of T cell-deficient mice were approximately 10% of the titers measured in the sera of wild type C57BL/6 mice, which mount a normal T cell-dependent antibody response [62].

Antibody responses to most protein antigens are CD4 T cell-dependent (TD), and involve germinal center formation, where the B cells undergo isotype switching and affinity maturation. TI antibody responses are categorically divided into two groups. TI-1 responses are usually induced by B cell mitogens such as LPS (which generate a polyclonal the B cell response), while TI-2 responses are elicited by highly organized repetitive antigens such as bacterial cell wall polysaccharides [67]. The repetitive structures of TI-2 antigens are thought to activate B cells by crosslinking the membrane-bound immunoglobulin B cell receptors, thereby delivering a strong activating signal that may override the need for other signals that are normally delivered by helper T cells [68]. TI-2 antigens can also defined by their inability to induce TI responses from Btk kinase-deficient B cells (Xid mutation) [69]. Btk-deficient B cells transferred into MPyV-infected SCID mice did not result in the generation of MPyV-specific antibodies indicating that MPyV induces a TI-2 antibody response [70]. The MPyV capsid, which is mainly comprised of 72 pentameric arrangements (capsomeres) of the VP1 protein, is a highly repetitive structure, thus resembling TI-2 antigens [71]. It is highly unusual, however, for a protein to elicit a TI-2 antibody response. Studies comparing TI antibody responses to inert non-repetitive (purified VP1 capsomeres), inert highly repetitive (purified VLPs) and live highly repetitive (infectious MPyV) forms of this antigen show that repetitiveness does contribute to generation of efficient IgM responses but is not sufficient to account for MPyV's ability to induce TI-2 IgG responses. VLPs induce only IgM and no IgG responses in T cell-deficient mice, whereas live MPyV elicits IgM and high titer IgG responses, suggesting that virus infection per se induces a variety of signals that may be essential for the generation of TI isotype-switched antibodies [62].

Although the signals and mechanisms involved in the TI induction of IgG by MPyV are not well understood, several factors are known to enhance TI antiviral B cell responses. While NK cells are not required for TI IgG secretion in response to MPyV, NK cells do enhance virus-specific IgG2a secretion by providing IFN- γ , an important factor that directs IgG class switching to the IgG2a isotype [70,72]. Engagement of CD40 surface molecules on B cells with CD40L on activated CD4 T cells forms an essential component of T cell help in immunocompetent mice. Surprisingly, B cells that lack CD40 expression produce significantly less TI IgG in response to MPyV infection, not only indicating that CD40 stimulation may be involved in TI antiviral antibody responses but that cells other than T cells can provide CD40L for these interactions. Studies suggest that B cell homotypic CD40-CD40L interactions could occur, thus enhancing the magnitude of TI IgG responses [73]. Additionally, complement activation seems to play a role in TI humoral responses to MPyV. Reconstitution of SCID mice with complement receptor-deficient B cells results in greatly reduced TI IgG antibody responses [74]. Thus, B cell activation may be augmented by viral antigen engagement of the B cell receptor coupled with complement components binding and cross-linking complement-specific receptors. Marginal zone (MZ) B cells, aptly named for their abundance in the splenic

marginal zone, respond quickly to pathogens, have high complement receptor expression, and are the source of most TI-2 antibody responses. Yet, recent work shows that both MZ B cells and follicular B cells respond to MPyV infection with TI IgG secretion when transferred into SCID mice (H.M. Guay, R.N. Mishra, and E. Szomolanyi-Tsuda, unpublished observations).

TI antibody responses may make significant contributions to the control of MPyV even in mice that have normal T cell populations. The onset of TI B cell responses precedes T cell activation and the generation of TD antibodies. Although the magnitude of the TI antibody responses may be relatively small, antibodies secreted at a very early phase of the infection could be crucial in slowing down the dissemination of this highly cytopathic virus, so that adaptive responses face a diminished virus load. TI humoral immune responses may also provide an additional layer of protection against the recrudescence of this persistent infection in the event that T cell responses become dysfunctional. Comparisons of MPyV-infected B cell-reconstituted SCID mice or TCR $\beta\delta$ KO mice to MPyV-infected SCID mice, which have no B cell responses, have revealed the importance of TI antibodies in reducing viral load ([60]; E. Szomolanyi-Tsuda, unpublished observations).

10. Long-term maintenance of antiviral antibody responses

Serological memory, defined as sustained antiviral antibody levels in the serum following infection, is crucial for protection against reinfection. In persistent infections, like MPyV, serological memory is also essential to check viral replication. Two forms of B cell memory are generated in TD humoral responses. B cells that have completed the germinal center reaction become either long-lived terminally differentiated plasma cells that migrate to the bone marrow where they continue to secrete antibodies, or they become memory B cells that reside in the spleens and secondary lymphoid organs and can rapidly reactivate upon antigen re-stimulation [75].

In MPyV-infected mice, VP1-specific serum IgG levels, after reaching a peak at 3–4 weeks post-infection, are maintained life-long [63]. Long-lived plasma cells (PC) are detectable in the bone marrow a few weeks after infection, and their number increase with time; by 18 months after infection the frequency of bone marrow (BM)-resident PC can be as high as $1-6 \times 10^3/10^6$ BM cells [63]. In mice lacking T cells (TCR $\beta\delta$ knockout (KO)) or MHC Class II molecules (I-A^b KO), MPyV-specific BM residing PC are not detected ([56]; E. Szomolanyi-Tsuda, unpublished observations), consistent with the notion that the formation of long-lived PC is T cell help-dependent. The formation of memory B cells is also thought to be TD, but data for this component of the B cell response to MPyV are scarce.

Recent studies have revealed that in addition to CD4 T cell help, innate immune signals are also essential for normal long-term antibody responses. Mice defective in MyD88, an adapter molecule involved in signal transduction of most toll-like receptors (TLR), and some cytokine receptor signals (IL-1R and IL-18R) have impaired long-term IgG responses to MPyV infection. MyD88 KO mice have decreased VP1-specific IgG levels after day 10 post-infection as well as a complete lack of MPyV-specific bone marrow PC, demonstrating a fundamental defect in the formation and/or maintenance of long-lived antibody secreting cells. Remarkably, this defect seems to be B cell intrinsic, as opposed to a consequence of impaired CD4 T cell help [63]. Combined with data showing that IL-1R and IL-18R KO mice have normal long-term antibody responses to MPyV, these findings suggest that intact MyD88-mediated TLR pathways in B cells are required for the generation of long-term humoral immunity to MPyV infection [63].

As mentioned above, wild type C57BL/6 mice have life-long humoral immunity to MPyV. On the other hand, MPyV-infected CD4 T cell-deficient mice, while having high TI IgM and IgG antibody levels during the acute phase of infection, have very diminished or even lack antiviral

serum IgG at later time points (1–6 months post-infection) [56]. This contrasts with data from MPyV-infected TCR $\beta\delta$ KO mice, which have high serum IgG levels maintained for many months after infection (E. Szomolanyi-Tsuda, unpublished observations). We speculate that the higher virus load in TCR $\beta\delta$ KO mice may trigger short-lived TI IgG responses by activating naïve B cells newly emerging from the bone marrow.

11. MPyV infection as a model for human polyomavirus pathogenesis

Over the last decade, the BK human polyomavirus (BKV) has emerged as a major cause of allograft failure following kidney transplantation [76]. The factors responsible for the increase in BKV-associated allograft nephropathy (BKVN) remain uncertain, although an association between the introduction of more potent new immunosuppressive agents (e.g., tacrolimus, mycophenolate mofetil) and BKVN is suspected [77]. Our understanding of the pathogenesis of BKVN following renal transplantation, as well as the development of effective therapies, has been limited due to the exquisite species specificity of the *Polyomaviridae* family that limits productive infection to their natural animal reservoir. It is conceivable that bystander injury to uninfected cells by activated virus-specific T cells or by an environment rich in proinflammatory cytokines and chemokines could augment the alloimmune response. Alternatively, allograft injury may be enhanced by the inability of recipient T cells to recognize viral peptides presented by donor-mismatched MHC molecules, resulting in unchecked viral replication and direct virus cytopathology. Allograft injury mediated by anti-donor T cells may also produce an inflammatory milieu that contributes to viral replication and ongoing tissue injury. Along the lines of the earlier discussion regarding the kidney as an immune-privileged organ, kidney-protective immunological tolerance may also be abrogated by the transient ischemia and reperfusion injury associated with organ procurement and transplantation and contribute to antigen-specific and bystander immunopathology. To help decipher the mechanisms of BKVN pathogenesis, an experimental model of MPyV infection following kidney transplantation in mice has been developed that reproduces key elements of BKV infection following renal transplantation [36]. Specifically, MPyV-inoculated recipients of fully MHC-mismatched kidneys suffer rapid graft injury and death, associated with high levels of allograft MPyV replication and a markedly elevated anti-donor T cell response. Whether kidney allograft loss is a consequence of direct cytolysis of infected cells, heightened alloimmunity, or bystander-associated inflammation are questions amenable to testing using this MPyV kidney transplant model.

12. Conclusions

Polyomaviruses are cytopathic viruses that cause silent, life-long infections in their natural host reservoirs. The importance of an intact immune system for containing polyomavirus infection and preventing its debilitating consequences is realized in immunocompromised individuals, such as those taking immunosuppressive drugs for kidney transplants or those with HIV/AIDS. The mouse model of polyomavirus infection has revealed that virus control is multifaceted, involving both humoral and cellular immune responses. Immunocompetent mice do not clear MPyV, with the virus likely persisting as infectious virus at low levels for the lifetime of the host. Because of this, MPyV infection is ubiquitous in the wild, as determined by serology [78].

Nevertheless, without one or more arms of the adaptive immune response, MPyV infection proceeds unchecked, a consequence being tumor induction. Studying MPyV infection has provided important insights into host mechanisms that control low-level systemic persistent viral infections. Our studies indicate that multiple components of the immune system contribute to limiting MPyV replication, including early induction of TI antibodies, recruitment of CD4 and CD8 T cells, and generation of TD humoral immunity. Each of these components has the

capacity to reduce viral load on its own, providing several layers of resistance to uncontrolled virus replication. Working together, they assure symptom-free survival with low-level viral persistence.

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Abbreviations

β₂m	β ₂ microglobulin
Ig	immunoglobulin
KO	gene knock-out
MHC	major histocompatibility complex
MPyV	mouse polyoma virus
MTV	mouse mammary tumor virus
PC	plasma cell/antibody secreting cell
TCR	T cell receptor
TD	T cell-dependent
TI	T cell-independent

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Table 1
Outcome of PyV infection in adult mice with various immunodeficiencies

Mouse strain ¹	Immune-deficiency	Survival past 3–5 weeks	Disease	Viral load ²	Reference
C57BL/6	none	100%	none	low	33,60
SCID	no T and B cells	0%	AMD ⁴	~10 ⁴ -fold higher than wild type	66
TCR βxδKO	no T cells	100%	tumors	~10-fold higher than wild type	60
μlg KO	no B cells	100%	none	N.D. ³	20,60
β ₂ m KO	no MHC class Ia and Ib	100%	tumors	~10-fold higher than wild type	14
K ^{b-/-} D ^{b-/-}	no MHC class Ia	100%	none	low	44
I-A ^b KO	no MHC class II	100%	none	low	56

¹ immunodeficient mice are all on the C57BL/6 genetic background

² determined by real time PCR of splenic DNA; E. Szomolanyi-Tsuda, unpublished results and indicated citations

³ not done

⁴ acute myeloproliferative disease