Immunity to Rotavirus Infection in Mice

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Recent findings from our laboratory regarding the immune response of mice to rotavirus (a mucosal pathogen) show that although in most situations an acquired (T or B cell or both) response is necessary for elimination of primary rotavirus infection, unidentified innate mechanisms can also play a role in some mouse strains. Similar to what is seen with many other viruses, CD8+ T cells appear to provide the first but not the exclusive mechanism that mediates clearance of a primary rotavirus infection. Antibodies are the critical mediators of prevention against rotavirus reinfection. Nonneutralizing IgA monoclonal antibodies directed against VP6 (an internal structural rotavirus protein) can mediate immunity against rotaviruses in vivo. Rotavirus-specific CD8+ T cells can mediate their antiviral effect in the absence of perforin, fas, or interferon-γ and are preferentially represented in the subset that expresses high levels of the enteric mucosal homing receptor α4β7.

Mice are susceptible to rotavirus-induced diarrhea only during the first 15 days of life; thus, it is almost impossible to study the active mechanisms that mediate immunity to disease. However, with selected strains of murine rotavirus, adult mice are as susceptible to infection as mouse pups [1]. Therefore, we have used rotavirus infection experiments in adult mice to study the determinants of protective immunity [1]. The experiments reviewed below were done using the adult model of rotavirus infection described by our laboratory and other laboratories [1, 2].

In adult mice, the appearance of an intestinal IgA anti-rotavirus immune response correlates with clearance of primary rotavirus infection [1]. Rotavirus-specific intestinal IgA persists for at least 1 year after primary infection, a time during which the mice are still completely protected from viral reinfection [1]. The kinetics and specificity of the rotavirus-specific antibody response have been studied in detail by use of an immunocytochemical staining assay of insect cells that were infected with recombinant baculovirus expressing selected rotavirus proteins [3, 4]. Both in serum and in the intestine, the rotavirus-specific antibodies are predominantly directed at the non-neutralizing major structural protein, VP6. The scope of viral proteins recognized by serum antibodies is broader than that recognized by intestinal antibodies. Antibodies to VP7, one of the most widely studied neutralizing rotavirus antigens, were detected in serum but not in suspensions of fecal samples. Unlike serum antibodies, which were long lived, intestinal antibodies to most proteins (VP6 is a notable exception) were short lived.

Most rotavirus vaccines currently being tested for use in humans are based on a Jennerian approach and use simian (RRV) or bovine (WC3) rotavirus reassortants as immunogens. We compared the relative efficiency of murine rotavirus (a homologous strain) versus simian rotavirus (RRV, a heterologous strain) to induce protective immunity in mice [5]. RRV could induce complete protection against infection with a murine virus only if given at high doses. This protection was correlated with the level of intestinal IgA but not serum IgG rotavirus-specific antibodies.

Some data from clinical and animal studies have indicated that neutralizing antibodies against the outer capsid rotavirus protein VP7 (G serotypes) were important in mediating protection against rotavirus infection [6]. For this reason, the current human RRV-based vaccine has been modified to include, in addition to RRV (G serotype 3), 3 reassortant viruses that contain human VP7 genes that determine the three other most common human rotavirus G serotypes. We compared the capacity of each of the four components (RRV and the 3 reassortant viruses) of the modified RRV-based rotavirus vaccine to protect mice from mouse rotavirus challenge [7]. All 4 viruses induced complete to near complete protection from challenge with the murine (G 3) virus. Mice that received the 3 reassortant viruses did not develop neutralizing antibodies against the challenge murine rotavirus. Thus, in mice, the induction of efficient protection does not require the induction of homotypic G-specific antibodies.

Effector Mechanisms that Mediate Clearance of Primary Rotavirus Infection

A combination of knockout mice deficient for selected immune-related genes, passive cell-transfer experiments, and im-
munodepletion of selected populations of lymphocytes by the administration of specific monoclonal antibodies has been used to delineate the effector mechanisms that mediate clearance of primary rotavirus [2, 8–10]. T cell– and B cell–deficient animals (Rag 2−/− and SCID mice) on BALB/c and 129/C57BL/6 backgrounds invariably become chronically infected with murine rotavirus [8, 11]. In contrast, 40% of SCID mice on a C57BL/6 background can clear primary rotavirus infection. Although in most cases an acquired immune response is necessary for clearance of a primary rotavirus infection, these results imply that uncharacterized innate mechanisms (that seem to be strain dependent) also can mediate rotavirus clearance. These innate mechanisms are uncharacterized. NK cells, which are known to have enhanced antiviral function in SCID mice [12], could possibly be mediating this anti-rotavirus effect.

Athymic nude mice, compared with immunocompetent control mice, clear a primary rotavirus infection with a short delay [10]. Clearance of infection in the nude mouse correlated with the appearance of a rotavirus-specific intestinal IgA response, suggesting that a T cell–independent antibody response might be responsible for virus clearance. Since intestinal IgA responses are believed to be strongly T cell dependent, this was an unexpected finding. To extend the results with nude mice, we did experiments with αβ, γδ (C57BL/6 background), and combined αβ/γδ T cell−/− mice (C57BL/6 × 129 background) [10]. Like nude mice, αβ and αβ/γδ T cell receptor−/− mice also cleared primary rotavirus infection with a short delay compared with the time for immunocompetent mice. These T cell receptor knockout mice also developed a rotavirus-specific intestinal IgA response. The intestinal IgA response in these T cell–deficient mice was roughly one-tenth that of immunocompetent mice, and it was directed exclusively at VP6.

In contrast, γδ T cell −/− mice cleared primary rotavirus infection without delay and produced normal amounts of intestinal IgA in a fashion similar to that for control mice. αβ T cell −/− mice depleted of γδ T cells by administration of a γδ T cell receptor–specific MAb cleared primary rotavirus infection like undepleted mice did and produced an anti-rotavirus intestinal IgA response of a magnitude similar to that for undepleted mice. These results indicate that αβ T cells are necessary for efficient clearance of primary rotavirus infection but that in their absence, a T cell–independent antibody response as well as an innate mechanism (as indicated by the results with SCID C57BL/6 mice, see above) can rapidly compensate and completely clear infection. γδ T cells do not seem to play any role in clearing primary rotavirus infection or in modulating the intestinal IgA anti-rotavirus antibody response.

Passive transfer of immune purified CD8+ T cells to chronically infected SCID mice cured them of rotavirus infection [11], and passive transfer of immune purified CD8+ T cells to mouse pups has also been shown to protect them from diarrhea induced by murine rotavirus [13]. To determine whether CD8+ T cells were important in the initial active clearance of primary rotavirus infection, we studied the infection in β2-microglobulin−/− mice that are deficient in class 1–restricted CD8+ T cells [8]. These mice cleared primary rotavirus infection like nude mice and αβ and αβ/γδ T cell receptor −/− mice did, indicating that αβ class 1–restricted CD8+ T cells were the cells needed for efficient and timely virus clearance. Immunocompetent C57BL/6 mice depleted of CD8+ T cells by administration of an anti-CD8 MAb also have a short delay in clearance of primary viral infection, confirming this conclusion [9, 10]. In contrast, C57BL/6 mice administered an anti-CD4 MAb cleared infection just like undepleted mice did despite a marked reduction in the production of virus-specific intestinal IgA. The finding of rotavirus-specific intestinal IgA in the CD4 cell–depleted mice suggests that the T cell–independent IgA found in the T cell knockout mice could also be present to some degree in immunocompetent mice.

The potential role of antibodies in mediating virus clearance was studied using a backpack tumor model with rotavirus-specific IgA hybridomas [14]. In mice, implantation of hybridomas secreting IgA antibodies specific for the non-neutralizing (internal structural protein) VP6 could mediate anti-rotavirus immunity. These antibodies were not active, however, when presented directly to the luminal side of the intestinal tract. These findings support the hypothesis that in vivo intracellular viral inactivation by secretory IgA during transcytosis is a mechanism of host defense against rotavirus infection.

Orally administered neutralizing monoclonal antibodies have been shown to passively protect mouse pups from heterologous rotavirus-induced diarrhea [15]. To address the role of antibodies in actively mediating clearance of primary rotavirus infection, we did studies with B cell–deficient JHΔ−/− mice [8]. Most of the mice cleared primary infection like immunocompetent control mice. Nonetheless, a small percentage of the B cell–deficient mice continued to shed low levels of viral antigen for several weeks after primary infection. When the mice were depleted of CD8+ T cells by administration of an anti-CD8 MAb, all of them shed high levels of viral antigen for as long as the anti-CD8 treatment persisted. In contrast, administration of a γδ T cell receptor–specific MAb had no effect on viral shedding. These results complement those described above with T cell–deficient mice and point to a complementary role of CD8+ αβ T cells and antibody in clearing primary rotavirus infection in mice.

**Effectors Mechanisms that Mediate Protection from Viral Reinfection**

Studies with the T or B cell knockout mice described above have highlighted a role for antibody in protection from viral reinfection. If the B cell–deficient JHD mice were challenged 6 weeks after primary infection, all could be reinfected, but compared with naive mice, they shed lower levels of viral antigen [8]. In contrast, β2-microglobulin and the αβ and αβ/γδ T cell receptor −/− mice were completely or almost completely resistant to re-infection at this time point [8, 10]. Immune-depletion exper-
iments showed that most of the partial protection against re-infection seen in the JHD mice was due to the presence of CD8+ T cells [16]. This partial protection mediated by the CD8+ T cells was almost complete up to 2 weeks after primary infection and diminished to almost nonexistent levels 8 months after primary infection. These results suggest that rotavirus-specific CD8+ memory T cells (probably because they are low in number or lack activation) generally do not prevent viral reinfection but limit the extent of the second infection by accelerating resolution.

Antiviral Effector Mechanism and the Phenotype of the Rotavirus-Specific CD8+ T Cells

To investigate the mechanism by which the CD8+ T cells were mediating rotavirus immunity, we studied rotavirus infection in perforin −/−, lpr (fas deficient), and interferon (IFN-γ) −/− mice [9]. Both knockout and lpr mice cleared rotavirus infection like immunocompetent control mice. The perforin and IFN-γ −/− mice, which were depleted of CD8+ T cells, had a short delay in virus clearance, as described above in T cell −/− mice and β2-microglobulin −/− mice. Thus, CD8+ T cells can mediate an anti-rotavirus effect in the absence of these molecules, which have been shown to be critical in many other viral model systems. This conclusion was further supported by experiments in which CD8+ T cells from an immune perforin −/− mouse, after passive transfer, could clear chronic rotavirus infection in Rag 2 −/− mice. Furthermore, JHD mice and perforin −/− mice depleted of IFN-γ by administration of a specific mAb cleared primary infection in a fashion similar to that in untreated mice. The antiviral mechanism used by rotavirus-specific CD8+ cells remains to be established.

An alternate cytokine through which CD8+ T cells could be mediating this effect is tumor necrosis factor (TNF): TNF released by CD8+ T cells has been suggested to act against cytomegalovirus [17] and to inhibit hepatitis B virus gene expression in hepatitis B virus transgenic mice [18]. In the transgenic hepatitis B model, the capacity of a perforin −/− CD8+ clone to abolish transgenic viral replication was partially inhibited by both anti–IFN-γ and TNF antibodies, and it was completely inhibited if the antibodies were administered simultaneously [18].

The integrin α4β7 has been shown to be an intestinal mucosal homing receptor that is present on lymphocytes capable of trafficking through the intestines and the intestinal lymphoid tissues. To address whether rotavirus-specific CD8+ T cells express this marker, we purified CD8+, CD44+ (as a memory marker), α4β7+, and α4β7− cells [19]. Upon transfer into chronically infected Rag 2 −/−, the α4β7− cells were more efficient at mediating virus clearance than the α4β7− cells. Although rotavirus-specific CD4+ T cells have not been shown to be essential for clearance of primary rotavirus infection in mice, they are definitely important in providing help for the development of the protective IgA response [10]. We have recently begun studying CD4+ rotavirus-specific human T cells and have determined that α4β7 (hi) memory (CD45RA−) CD4+ T cells display much greater reactivity to rotavirus than do α4β7+ memory or naive (CD45RA+) CD4+ T cells [20]. In contrast, α4β7+ memory cells were the predominant population responsive to mumps antigen after intramuscular vaccination [20]. Taken together, these studies support the hypothesis that both in humans and mice under physiologic conditions, the expression of tissue-selective homing receptors may help segregate intestinal from systemic T cell responses.

Conclusion

The direct relevance of the studies described herein to the human situation remains to be established. Our initial studies with human CD4+ T cells suggest that some important similarities between the immune response against rotavirus in humans and mice does exist. In addition to being a useful model to help develop better anti-rotavirus vaccines, the murine model of rotavirus infection will continue to provide insights into the immune response of the enteric mucosa. The existence of a completely T cell–independent antiviral IgA response, viral immunity mediated by a non-neutralizing IgA antibody, and the atypical antiviral mechanism used by CD8+ T cells that mediate immunity to rotavirus are all worthwhile to lead to when studying the enteric mucosal responses to other pathogens.

References

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