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Experimental Intestinal Reovirus Infection of Mice: What We Know, What We Need to Know

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Abstract

Reovirus, a member of the Reoviridae family, is a ubiquitous virus in vertebrate hosts. Although disease caused by reovirus infection is for the most part mild, studies of reovirus have been particularly valuable as a model for understanding the local host response to replicating foreign antigen in intestinal and respiratory sites. In this article, a brief overview is presented of the basic features of reovirus infection, as will the host's humoral and cellular immune response to during the infectious cycle. New information regarding the interactions and involvement of immune response molecules during reovirus infection will be presented based on multiple analyte array studies from our laboratory.

Keywords

Analyte; Effector; Intestine; IEL; Viral

Introduction

Reovirus is a non-enveloped virus made up of segmented double-stranded RNA inside a double-layered capsid shell [1,2]. Three serotypes have been defined for reovirus, designated serotype 1 (prototype Lang strain), serotype 2 (prototype Jones strain), and serotype 3 (prototype Dearing strain). The outer capsid consists of both structural and non-structural polypeptides [3,4]. The $\sigma 1$ hemagglutinin, a structural capsid molecule, has been linked to many virus associated activities, including host cell attachment, viral neutralization by antibody, recognition by cytotoxic T cells, and tissue tropism and pathogenesis of reovirus-mediated diseases [5–16]. Core proteins consist of transcriptases and replicases used in viral replication.

Virus infectivity by reovirus spans a wide range of vertebrate species [17–19], and the virus has served well as an experimental system for studying the dynamics of acute enteric and respiratory virus infection in laboratory animals. Differences have been reported in regional areas of infectivity in the small intestine using reovirus serotype 1 versus serotype 3, with serotype 1 primarily infecting epithelial cells and serotype 3 infecting intestinal intraepithelial lymphocytes (IELs) [20–25]. In both cases, however, entry of the virus appears to occur through Peyer's patches, most likely via M cells [26]. Following oral infection, the virus may spread to mesenteric lymph nodes [27] and may disseminate and penetrate into many tissues, including the liver [28,29], pancreas [5,30], and endocrine tissues [31], as well as muscle [32] and heart

tissue [33–35]. Age-associated susceptibility to reovirus serotype 3 leading to fatal encephalitis has been reported, the effect of which does not appear to be mediated by immunological factors but most likely reflect developmental conditions of neural tissues [36]. An autoimmune diabetes has been described following reovirus infection in mice [37], and human pancreatic beta cells are susceptible to in vitro reovirus infection [38].

Humoral and Cellular Immunity to Reovirus

Both neutralizing and non-neutralizing antibodies develop following reovirus infection [39–42]. The hemagglutinin $\sigma 1$ protein is the primary target molecule of neutralizing antibodies [43–45] as seen from studies using cloned *S1* gene products recognized by monoclonal and polyclonal antibodies [45]. Moreover, cloning of *S1* gene cDNA in *E. coli*, resulting in a fusion protein with hemagglutinating activity and binding capacity to L cells [46], and analysis using mutant $\sigma 1$ proteins, revealed that viral domains differ with regard to their tissue binding capacities and hemagglutinating activities [46]. Likewise, site-directed mutagenesis of the *S1* gene has identified conserved attachment sites of the $\sigma 1$ protein with the location of greatest activity in the c-terminal portion [47]. Antibodies to the $\sigma 1$ protein have been shown to prevent the spread of reovirus serotype 3 to the CNS and to protect against lethal disease [44,48]. In studies using reovirus serotypes 1 and 3, infection of the P388D1 macrophage cell line was enhanced in the presence of non-neutralizing antibody or with neutralizing antibody at low concentrations, an effect presumably due to endocytosis of virus-antibody complexes following Fc receptor capture by cells [49]. Recent studies have demonstrated a role for IgG and IgA antibodies directed to $\sigma 1$ protein in *in vivo* protection against Peyer's patch (PP) infection when antibody was administered orally with infectious virus. In contrast, only IgA effectively blocked infection when secreted from subcutaneous hybridoma tumors, a system that would more closely mimic systemic antibody dissemination [50]. The route of infection influences the immunoglobulin isotype generated. This is seen in studies in which oral reovirus exposure leading to infection of PP stimulated IgA synthesis while intradermal virus infection elicited IgG from lymph node cells [39]. IgG was present in the serum of mice following either oral or intradermal infection [39].

Finberg and colleagues provided the first evidence that MHC-restricted, reovirus-specific cytotoxic T lymphocytes (CTL) generated from reovirus-infected mice infected with reovirus serotypes 1 or 3 [8], and to demonstrate that anti-reovirus CTL respond to the $\sigma 1$ hemagglutinin [8]. Subsequent studies have confirmed a role for CTL in the anti-reovirus immune response [42,51–54]. However, there appears to be a significant degree of cross-reactivity of CTL raised to serotype 1 or 3 [52–54]. Additionally, studies of CTL specificities using vaccinia virus-reovirus recombinant constructs indicate both strain specific and cross-reacting CTL directed to the $\sigma 1$ non-structural capsid protein [52].

TCR $\alpha\beta$ T cells appear to account for most reovirus-specific CTL; the role of $\gamma\delta$ T cells in the immune response to reovirus has not been thoroughly explored. T CTL V β repertoire appears to be skewed in favor of V $\beta 6$, V $\beta 12$, and V $\beta 17$, although V $\beta 2$, V $\beta 7$, V $\beta 9$, and V $\beta 14$ also may participate in the response to reovirus [55,56]. Interestingly, the J β gene segment of V $\beta 6$ TCR clones expanded in SCID mice showed more restricted J β gene usage in orally-infected mice versus systemically-infected animals [55], implying that repertoire selection of anti-reoviral T cells is dictated in part by the route of viral entry. Using a germinal center/T cell marker (GCT), London and colleagues reported an increase in GCT+, Thy-1+, CD8+ IELs and lamina propria lymphocytes (LPL) bearing CD11c, a marker of T cell activation in the gut [57], in mice infected intraduodenally with serotype 1 reovirus T1L [58]. Modest differences also were noted in the cytotoxic activities mediated via perforin, FasL, and TRAIL for PP lymphocytes, IELs, and LPLs [58]. Local dendritic cells play a clear role in determining the outcome of virus delivery within mucosal tissues, leading either to immune tolerance or lymphoid activation

[59–61]. Moreover, CD11c+, CD8α+ DCs in PP subepithelial dome contribute to CD4+ T cell priming by viral antigen cross-presentation [59].

A role for the immune response in curtailing the reovirus infectious process is also evident from studies using severe-combined immunodeficient (SCID) mice, which develop a systemic reovirus infection leading to death of the host [62]. Protection of SCID mice can be achieved upon adoptive transfer of PP cells from immune animals [62]. Interestingly, the role for NK cells during virus infection is unclear. Given that SCID mice, which have significant numbers of NK cells despite a lack of mature functional T cells, are susceptible to reovirus infection [62], it would appear that adaptive immune elements play a necessary and indispensable role in curtailing the spread of virus. Yet, studies using human peripheral blood mononuclear cells demonstrate *in vitro* lysis of reovirus-infected K562 NK-sensitive target cells, the activity of which is influenced by IL-15 [63]. Moreover, reovirus infection is a potent inducer of NK cell activity in mice [64], and some anti-reovirus CTL express the asialo GM1 marker commonly found on NK cells; however, those cells were not lytic for NK-sensitive target cells [65], suggesting that they conform most closely to conventional CTL. Thus, it appears that both NK cells and CTL are needed for the development of an adequate immune response, with NK cells being required early during the infectious cycle and the classical adaptive cellular immune response mediated by CTL being essential late in the cycle. In the absence of either of those, viral infection is not adequately controlled.

Immunoregulatory and Effector Cytokines during Reovirus Infection – Analysis using Multiple Analyte Arrays

Information remains incomplete about the role played by immune regulatory cytokines and chemokines in the local host immune response to reovirus infection. Studies of cytokine production by mice following oral infection with serotype 1 reovirus characterize elevated levels of gene expression for IFN-γ in PP lymphocytes and IELs, and synthesis of IL-5, IL-6, and IFN-γ from cultured PP cells of reovirus infected mice [66]. Differences also exist in the levels of IFN-γ produced by lymph node cells following intradermal infection, with C3H mice being high producers and BALB/c, C57BL/6, and B10.D2 mice being low responders [39]. IFN-γ and IL-12 mRNA levels increase in draining popliteal or mesenteric lymph nodes following footpad or oral infection with reovirus serotype 1, respectively [67].

In an effort to gain a more comprehensive understanding of the involvement of regulatory and effector molecules in the response to reovirus infection, we initiated a series of studies aimed at defining changes that take place in cytokine and chemokine activities in intestinal tissues using a system for simultaneously evaluating twenty-two immune regulatory and effector immune response molecules. Mice were infected with 10^{7.5} reovirus type 3 Dearing strain by oral gavage. Previous studies from our laboratory using real-time PCR indicate that virus infection peaked in the small intestine between days 4–7 post infection [68]. On day 4 post-infection, IELs were isolated and cultured overnight in serum-free tissue culture medium. Cell-free supernatants were recovered and reacted with commercial membranes (RayBiotech, Inc; Norcross, GA) that measure the activity of the twenty-two analytes shown in Table 1. The results of these findings are shown in Fig. 1 for IELs from non-infected mice and from mice after 4 days of reovirus infection. Note the increase in activity of a number of analytes in IELs from virus-infected mice compared to IELs from non-infected animals. After normalization of the data using positive controls included with the membranes, the data are displayed graphically as seen in Fig. 2, allowing direct comparisons and quantification of the changes in analyte values that cannot be directly determined by visual inspection but are quantifiable based on optical density, thus revealing numerous analyte changes during infection.

What do these findings tell us about the natural intestinal immune response to reovirus? It is clear that virus infection leads to a complex but selective pattern of expression of immune response molecules produced by intestinal IELs. This can be seen in Table 2, in which analytes have been grouped based on whether they had a >2-fold or <2-fold change in analyte levels relative to that of normal IELs. Of the analytes showing elevated changes, several are characterized by their strong inflammatory activities (IL-6, IL-12, IFN- γ), or as analytes that are used in T cell activation, e.g., IL-17 and SCF. Upregulation of activity for colony-stimulating factors (G-CSF and GM-CSF) and monocyte chemoattractant proteins (MCP-1 and MCP-5) point to a process of mobilization and/or cell proliferation of dendritic cells, monocytes, and macrophages during the height of infection. Stem cell factor (SCF) would be used in the expansion/development of intestinal T cells during infection [69]. Collectively, the composite effects of those analytes in the host response to enteric virus infection would predictably lead to a strong cell-mediated immune response on day 4 of infection, a time when virus is wide-spread [68].

An additional novel finding from these studies pertains to the high level of IL-10 production by reovirus-infected IELs compared to IELs from non-infected mice (Table 2). Interestingly, other studies have described suppressed IL-10 activity in PP of mice infected with reovirus serotype 1 [67]. Those differences may reflect the fact that our studies were done using IELs from intestinal tissues after removal of PPs, or alternatively that virus strain differences influence the outcome of IL-10 production in the small intestine. Nonetheless, it may be of value to re-evaluate the relative contributions of PP cells and IELs to the overall host response to reovirus. Regardless, the data shown in Table 2 for IL-10 imply that the immune response to virus proceeds in the face of a significant level of immunological control. Although this may appear to be intuitively obvious, studies that examine regulatory and effector cytokines individually, without the benefit of a holistic integrative analysis provided by multiple analyte determinations, are likely to miss this connection. Indeed, further studies using multiple analyte systems will undoubtedly provide new insight into the totality of the immune response at the level of the intestinal mucosa.

Information derived from the nine analytes that had <2-fold changes compared to normal mice are equally revealing. Both IL-2 and IL-4, cytokines that exert proliferative effects on lymphocytes, were only modestly elevated during infection — at least at day 4 post-infection. On the one hand, this could be indicative of a temporal effect such that the greatest level of proliferation mediated by IL-2 and IL-4 may have already occurred prior to day 4. It should be noted that IL-4 was present in significant levels in non-infected mice (Fig. 1 and Fig. 2), possibly indicating an ongoing process of IL-4 synthesis in the small intestine due to a homeostatic process of T cell and/or B cell expansion in the small intestine. Likewise, RANTES (CCL5) expression, though not appreciably elevated in virus-infected mice, was nonetheless one of the analytes having the greatest activity in array blots of both non-infected and infected mice (Fig. 1 and Fig. 2). That pattern is consistent with RANTES being synthesized by resting T cells and with the elevation of RANTES secretion following T cell activation. If IELs are activated or partially-activated T cells, as has been predicted [70–72], background levels of RANTES would already be high, masking changes that occur in RANTES production by anti-viral T cells during the infection cycle.

Where to from Here?

It will be particularly interesting to conduct similar analyte expression studies at temporal intervals during the course of reovirus infection, and to conduct analyte studies within selected areas of the intestine (duodenum, jejunum, ileum, and regional Peyer's patches). An obvious void in our knowledge of the reovirus infectious process concerns the lack of information about the natural history of the virus throughout the intestine following exposure. Where, for

example, does the virus reside following oral ingestion? Is the virus uniformly or randomly distributed in intestinal tissue sites? Are there changes in the location of virus infection across time? If so, what accounts for those changes? What is the nature of the local site-specific immune response as virus infection proceeds? These are a few of the fundamentally-important issues that bear directly on issues pertaining to the manipulation of the host immune response through vaccination or therapy, and they are central to our understanding of how the intestinal immune system responds to a replicating foreign antigen in general.

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Fig. 1. Analysis of twenty-two immune response analytes produced by IELs from non-infected mice and IELs from mice 4 days after reovirus infection. Note the increase in synthesis of analytes in infected mice compared to non-infected animals. See Table 1 for identification of analytes in blot grids.

Fig. 2.

Comparison of data from Fig. 1 after normalization using the positive and negative control values provided with the blotting membranes.

Table 1

Template for Protein Array Grids

	a	b	c	d	e	f	g	h
1	Pos	Pos	Neg	Neg	GCSF	GM-CSF	IL-2	IL-3
2	Pos	Pos	Neg	Neg	GCSF	GM-CSF	IL-2	IL-3
3	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12	IL-12p70	IL-13
4	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12	IL-12p70	IL-13
5	IL-17	IFN- γ	MCP-1	MCP-5	RANTES	SCF	sTNFRI	TNF- α
6	IL-17	IFN- γ	MCP-1	MCP-5	RANTES	SCF	sTNFRI	TNF- α
7	TPO	VEGF	Blank	Blank	Blank	Blank	Blank	Pos
8	TPO	VEGF	Blank	Blank	Blank	Blank	Blank	Pos

Table 2

Changes in Analyte Levels in Intestinal IELs from Day 4 Reovirus-infected Mice

Fold change >2.0		Fold change <2.0	
Analyte	Fold	Analyte	Fold
GCSF	2.1	IL-2	1.3
GM-CSF	3.5	IL-3	1.2
IL-5	3.0	IL-4	1.1
IL-6	2.2	IL-13	1.7
IL-9	2.6	RANTES	1.1
IL-10	7.3	sTNFR1	1.8
IL-12	4.4	TNF α	1.7
IL-12p70	3.3	TPO	1.2
IL-17	4.0	VEGF	1.0
IFN- γ	5.9		
MCP-1	3.0		
MCP-5	3.1		
SCF	2.6		