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Rotavirus acceleration of murine type 1 diabetes is associated with increased MHC class I-restricted antigen presentation by B cells and elevated proinflammatory cytokine expression by T cells

Jessica A. Pane, Nicole L. Webster, Christel Zufferey[1], Barbara S. Coulson*

Department of Microbiology and Immunology, The University of Melbourne, Parkville, Victoria 3010, Australia

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ABSTRACT

Rotavirus infection has been proposed to enhance progression towards type 1 diabetes in at-risk children. Rhesus monkey rotavirus (RRV) accelerates diabetes onset in non-obese diabetic (NOD) and T cell receptor transgenic NOD8.3 mice. Infected NOD mice show virus spread to pancreatic lymph nodes (PLN) and mesenteric lymph nodes (MLN), induction of a serum T helper 1-biased specific antibody response and proinflammatory cytokine mRNA expression in PLN and islets. Here, we analysed the effects of RRV infection on intestinal responses and the activation of antigen presenting cells (APC), T cells and B cells in PLN, MLN, spleen and islets. Diabetes acceleration by RRV was associated with minimal immune activation in Peyer's patches. Increased proinflammatory cytokine expression by APC, including dendritic cells, was observed exclusively in the PLN, while cytokine expression by T cells was detected in islets, PLN, MLN and spleen. RRV infection of NOD8.3 mice increased IFNγ expression by $CD8^+$ T cells, which primarily recognise an islet autoantigen. A peptide corresponding to RRV VP7 amino acids 5–13, with sequence similarity to this islet autoantigen, did not induce activation or proliferation of NOD8.3 mouse T cells. RRV infection of NOD mice elevated B cell MHC I expression in PLN and MLN, and increased the B cell-mediated proliferation of islet antigen-specific $CD8^+$ T cells. These studies demonstrate that RRV infection of NOD mice activates APC, T cells and B cells at sites where autoreactive lymphocytes accumulate, in association with proinflammatory cytokine expression and an increased capacity to present antigen. Taken together with previous findings, these data support a possible role for bystander activation in type 1 diabetes acceleration by RRV.

1. Introduction

Type 1 diabetes is a chronic autoimmune condition characterised by destruction of pancreatic insulin-secreting β cells. Non-obese diabetic (NOD) mice spontaneously develop type 1 diabetes with age, and are widely used to model the human disease. Extensive islet infiltrates primarily consisting of T cells, B cells, dendritic cells (DC) and macrophages develop in all NOD mice by 10 weeks of age (Kikutani and Makino, 1992). Activation of these T cells is mediated by autoantigen presentation on B cells and DC (Calderon et al., 2008; Marino et al., 2012). As diabetes progresses, the proportion of activated immune cells and expression of cytotoxicity markers increases (Diana et al., 2013; Marino et al., 2008; Rabinovitch et al., 1995). Furthermore, cytotoxic T lymphocytes in the pancreas show increased levels of cytotoxicity markers, including proinflammatory cytokines and granzyme B, compared with PLN T cells (Graham et al., 2011). Multiple mechanisms for β cell death have been described (Cnop et al., 2005), including exposure to proinflammatory cytokines (Suk et al., 2001; Wachlin et al., 2003). The onset of type 1 diabetes occurs when β cell mass is reduced below a critical level. Approximately 60–80% of female NOD mice develop type 1 diabetes by 30 weeks of age (Kikutani and Makino, 1992). The age at diabetes onset can vary substantially between mice, and typically the extent of cellular activation in the PLN and islets is more variable than in non-diabetes prone mouse strains.

Viral infections are widely studied as environmental modulators of diabetes initiation and progression (van Belle et al., 2011). Pancreatic infection, T cell molecular mimicry and bystander lymphocyte activation are the major mechanisms proposed for virus-induced diabetes acceleration (Coppieters et al., 2012a,b). Apart from the effects of direct β cell infection and lysis,

Abbreviations: RRV, rhesus monkey rotavirus; PLN, pancreatic lymph nodes; MLN, mesenteric lymph nodes.

* Corresponding author at: Department of Microbiology and Immunology, Gate 11, Royal Parade, The University of Melbourne, Melbourne, Victoria 3010, Australia. Tel.: +61 3 8344 8823; fax: +61 3 9347 1540.
E-mail address: barbarac@unimelb.edu.au (B.S. Coulson).
[1] Current address: Infectious Diseases and Microbiology, Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia.

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pancreatic infection and molecular mimicry involve T cell receptor (TCR)-mediated activation of autoreactive T cells. In contrast, as bystander activation is TCR-independent, it is expected to encompass polyclonal activation of T and B cells through the expression of proinflammatory cytokines secreted by virus-activated DC. As bystander activation would require pre-existing autoreactive cells to accelerate diabetes onset, it would be expected to occur in the islets, PLN and possibly MLN, where these cells accumulate (Jaakkola et al., 2003). Additionally, intestinal infection and inflammation are increasingly being associated with diabetes progression (Hara et al., 2012; Lee et al., 2010; Oikarinen et al., 2012; Snell-Bergeon et al., 2012).

Infection of children genetically at-risk of developing type 1 diabetes with members of the virus family *Reoviridae*, genus *Rotavirus* has been associated with increased levels of islet autoantibodies, which mark progression towards diabetes (Honeyman et al., 2000; Lempainen et al., 2012). Additionally, MHC II-restricted T cell molecular mimicry between rotavirus viral protein 7 (VP7) and islet autoantigen-2 (IA-2) has been reported using human peripheral blood mononuclear cells (Honeyman et al., 2010).

NOD mice infected with rhesus monkey rotavirus (RRV) at 12 weeks of age show accelerated diabetes onset (Graham et al., 2008). As diabetes development is unaltered in 4 week-old NOD mice given RRV (Graham et al., 2007) and 12 week-old NOD mice given porcine rotavirus CRW-8 (Pane et al., 2013), pre-existing autoimmunity and virus strain-specific factors are required for further autoimmune exacerbation by rotavirus. Diabetes acceleration by RRV does not require virus spread to the pancreas but instead correlates with production of serum anti-rotavirus antibodies biased towards a Th1-specific response, and proinflammatory cytokine mRNA expression in PLN and islets (Graham et al., 2008; Pane et al., 2013). Therefore, molecular mimicry, intestinal inflammation and bystander activation are potential mechanisms for diabetes acceleration by RRV.

A similar acceleration of diabetes development results from RRV infection of TCR-transgenic NOD8.3 mice, in which a majority of $CD8^+$ T cells are specific for an immunodominant epitope in the islet-specific glucose 6-phosphatase catalytic subunit-related protein (IGRP), a type 1 diabetes autoantigen (Graham et al., 2008; Lieberman et al., 2003). T cell autoimmunity to IGRP is an essential requirement for diabetes development in these mice (Krishnamurthy et al., 2008). $CD8^+$ T cells directed to this IGRP epitope also are found in PLN and islets of NOD mice, and contribute to their diabetes development (Marino et al., 2012; Young et al., 2009). NOD8.3 mice thus provide a model for detection of T cell molecular mimicry between IGRP and rotavirus. No studies addressing this area have been reported.

RRV infection does not cause overt gastrointestinal symptoms in adult NOD mice, and little is known about the intestinal immune responses of these mice to rotavirus (Graham et al., 2008; Pane et al., 2013). Infectious RRV and RRV antigen are present intestinally, although they are more frequently detected in MLN where RRV is harboured by antigen presenting cells (APC) but not T or B cells (Graham et al., 2008; Pane et al., 2013). MLN APC containing intracellular RRV show signs of maturation (Pane et al., 2013). RRV infection does not alter the number or frequency of intestinal $CD8^+$ TCRαβ intraepithelial lymphocytes (Webster et al., 2013). B cell proliferation and activation is common in Peyer's patches and MLN of RRV-infected adult non-diabetes prone mice (Blutt et al., 2002). However, immune cell activation at these sites in adult NOD mice following RRV infection has not been studied. Understanding the ability of RRV to induce intestinal inflammation in adult NOD mice is important as this could contribute to their accelerated diabetes onset following RRV infection.

Infectious RRV and RRV antigen also are present in PLN of adult NOD mice following RRV infection (Pane et al., 2013). Gut-derived antigens are preferentially presented to T cells in the PLN of NOD mice, implying that gastrointestinal antigen may be transported to the PLN and induce local inflammatory responses at this site in preference to the small intestine (Turley et al., 2005). Increased TNF and IFNγ mRNA levels in the PLN of RRV-infected NOD mice support the possible activation of T cells (Pane et al., 2013). However, cytokine expression by these cells has not yet been demonstrated. RRV infection of male NOD mice increases the proportion of B cells in islets and PLN, also suggesting that RRV infection activates these cells (Graham et al., 2008). RRV association with and maturation of APC in the lymph nodes of NOD mice could also reflect the ability of RRV to induce APC-mediated bystander lymphocyte activation at these sites. Therefore, analysis of B and T cell activation in the lymph nodes is required.

In this study, intestinal responses and the activation of APC, T cells and B cells were analysed in the lymph nodes, islets and spleen in RRV-infected adult NOD mice. Limited immune cell activation occurred in Peyer's patches. However, IFNγ and TNF expression by T cells and APC was increased extraintestinally. RRV infection also activated splenic $CD8^+$ T cells isolated from NOD8.3 mice, although stimulation with a possible IGRP epitope mimic (an RRV VP7 peptide) did not activate these T cells. Rather, RRV infection of NOD mice upregulated B cell MHC I expression, leading to increased antigen presentation to islet-specific $CD8^+$ T cells. These studies support a role for bystander activation in type 1 diabetes acceleration by RRV.

2. Results

2.1. RRV infection of NOD mice induced minimal immune cell activation in the Peyer's patches

Adult RRV-infected NOD mice show no signs of diarrhoea at any time and no virus excretion later than 7 days post infection (Graham et al., 2008). The effect of RRV infection on intestinal inflammation was determined using Peyer's patch cells isolated from mock- and RRV-inoculated adult females on days 3 and 7 post inoculation and intestinal intraepithelial lymphocytes (IEL) isolated on days 2, 5 and 7 post inoculation. Total cell numbers obtained from Peyer's patches were similar between mock- and RRV-inoculated mice (Fig. 1A, $p > 0.05$), as were the frequencies of APC ($CD3^-CD19^-MHCII^+$), B cells, $CD4^+$ T cells and $CD8^+$ T cells (data not shown, $p > 0.05$). This contrasts with RRV infection in non-diabetes prone adult mice, where Peyer's patch cell numbers increase during this period (Blutt et al., 2002). IEL numbers in adult NOD mice also were unchanged by RRV infection (Fig. 1B, $p > 0.05$). The proportions of $CD4^+$ TCRαβ T cells, $CD8^+$ TCRαβ T cells and $CD8^+$ TCRγδ T cells in IEL were unaltered (data not shown, $p > 0.05$). However, $CD8^+$ TCRγδ T cell numbers within IEL were reduced from a mean ± SEM of $1.2 \times 10^6 \pm 3 \times 10^4$ in mock-inoculated adult mice ($n = 2$) to $7.3 \times 10^5 \pm 2 \times 10^4$ in mice given RRV ($n = 2$; $p = 0.005$) at day 2 post inoculation. This contrasts with infant NOD mice, where numbers and proportions of intestinal $CD8^+$ TCRαβ T cells were increased by RRV infection, and $CD8^+$ TCRγδ T cells were unchanged (Webster et al., 2013). Overall, RRV infection of adult NOD mice did not increase immune cell numbers in the small intestine, differing from other murine models of RRV infection.

In adult non-diabetes prone mice, RRV infection induces DC and B cell, but not T cell, activation in the Peyer's patches up to day 3 post infection (Blutt et al., 2002; Lopez-Guerrero et al., 2010). In NOD mice, Peyer's patch B cell MHC I expression was unaltered at day 3 post infection, but elevated at day 7 (Fig. 1C; paired *t*-test, $p = 0.0110$). B cell expression of MHC II, CD86 and CD80 was unchanged (data not shown; $p > 0.05$). Additionally, Peyer's patch APC showed unaltered expression of MHC I (Fig. 1C, $p > 0.05$), and of