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ScienceDirect
Cellular Immunology 246 (2007) 8–16
Cellular Immunology
www.elsevier.com/locate/ycimm

Mucosal lymphatic-derived γδ T cells respond early to experimental *Salmonella* enterocolitis by increasing expression of IL-2Rα

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Received 3 April 2007; accepted 26 April 2007
Available online 15 June 2007

Abstract

To better understand the roles of γδ T cells in mucosal infection, we utilized *Salmonella enterica* serovar *Typhimurium* (*Salmonella* serovar *Typhimurium*) infection in cattle as it closely approximates *Salmonella* serovar *Typhimurium*-induced enterocolitis in humans. Protein and gene expression in αβ and γδ T cells derived from lymphatic ducts draining the gut mucosa in *Salmonella* serovar *Typhimurium*-infected calves were analyzed. In calves with enterocolitis, general gene expression trends in γδ T cells suggested subtle activation and innate response, whereas αβ T cells were relatively quiescent following *Salmonella* serovar *Typhimurium* infection. An increase in IL-2Rα expression on γδ T cells from infected calves and results from *in vitro* assays suggested that γδ T cells were primed by *Salmonella* serovar *Typhimurium* LPS to better respond to IL-2 and IL-15. Together with gene expression trends *in vivo*, these data support early priming activation of target tissue γδ T cells during *Salmonella* serovar *Typhimurium* infection.

Keywords: γδ T cells; Mucosal; IL-2Rα; Cell proliferation; *Salmonella* serovar *Typhimurium*; Gene expression; Lymphatic; Priming

1. Introduction

Depending on the serotype and host, *Salmonella* can cause bacteremia, typhoid fever or enterocolitis. Bacteremia induced by infection with *Salmonella enterica* serovars Choleraesuis (swine adapted) and Dublin (bovine adapted) is relatively rare in humans [1]. Typhoid fever caused by *S. enterica* serovar Typhi in humans is virtually eradicated in the US, but remains a problem in regions of the developing world with poor sanitation practices [1]. In contrast, *Salmonella*-induced enterocolitis in humans, primarily caused by *S. enterica* serovar *Typhimurium* (*Salmonella* serovar *Typhimurium*), is extremely common and is the most common cause of death by food-borne illness in the US [1,2]. The role of T cells in infection with *Salmonella* species has largely been investigated in mice [3]. However, while infection of mice with *Salmonella* serovar *Typhimurium* is an excellent model for human typhoid fever, it does not induce enterocolitis [1,3]. In contrast, *Salmonella* serovar *Typhimurium* infection in calves induces enterocolitis that closely mirrors disease in humans and is also the most common *Salmonella* strain associated with ill cattle in the US [1].

γδ T cells localize to the gut mucosa in all animals, including humans, express the γδ TCR of limited diversity, recognize unprocessed antigen, and respond rapidly to infection, in part by rapid recruitment to infected sites [4,5]. Whereas αβ T cells appear to be more important than γδ T cells in the clearance of *Salmonella* serovar *Typhimurium* in the typhoid fever model in mice [1,3], the roles of γδ T cells in the gut mucosa of a highly relevant model of enterocolitis have not been investigated. Since γδ T cells localize to the gut mucosa and are thought to participate in innate immune responses, they are likely involved in early protective responses to *Salmonella* serovar *Typhimurium* infection [6]. The phenotype of T cell populations in blood, peritoneal fluid, or lymphoid tissues, (the

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doi:10.1016/j.cellimm.2007.04.006

sources of lymphocytes in several murine and human investigations [3,7,8]), does not necessarily reflect that of those responding to initial infection in the target tissue. We hypothesized that γδ T cells derived from the gut mucosal lymphatic ducts provide an early innate response during *Salmonella* serovar *Typhimurium* enterocolitis. To address this hypothesis, cells in lymphatic fluid draining from the intestine were collected at early intervals during *Salmonella* serovar *Typhimurium*-induced enterocolitis in naïve bovine calves. T cells were analyzed by FACS and microarray to determine their protein and gene expression phenotypes. Among other indicators of activation, IL-2 receptor α (IL-2Rα) transcripts and protein increased in expression on γδ T cells during *Salmonella* serovar *Typhimurium* infection *in vivo*, whereas αβ T cells appeared minimally stimulated. In an *in vitro* functional assay, highly purified γδ T cells stimulated with *Salmonella* serovar *Typhimurium* LPS had enhanced proliferation in response to IL-2 and IL-15. Our results indicate an early priming activation of γδ T cells, relative to a less dramatic early role of αβ T cells in an infrequently studied but highly relevant model of *Salmonella* serovar *Typhimurium*-induced enterocolitis.

2. Materials and methods

2.1. Surgery and experimental infection

Four- to six-week old calves with no evidence of prior *Salmonella* infection were used for the surgery and infection. Catheters were inserted into the mesenteric efferent lymphatic vessel following standard surgical procedures. Calves recovered from surgery for approximately 20 h, then the time 0 lymphatic fluid and blood were collected just prior to infection with 4.7×10^7 to 4×10^8 CFUs of a natural calf isolate of *Salmonella* serovar *Typhimurium* (Type O, Group B) or mock infection with an equivalent volume of sterile Luria broth (LB) media. Prior to infection, 100 μl of *Salmonella* serovar *Typhimurium* stock was added to 5 ml of LB media and shaken for 6 h at 37°C. Bacterial cell growth was determined by absorbance reading and comparison to a standard growth curve for the same strain. CFU counts were verified by plating serial dilutions of bacterial suspensions on LB agar plates. Blood and lymphatic fluid were collected at intervals post-infection during which the calves were closely monitored. Enterocolitis was defined as fever (>105 °F) and diarrhea between 24- and 48-h post-infection. At this time fecal samples were submitted to the Montana State Veterinary Diagnostic Service and those from experimentally infected calves tested positive for the input strain of *Salmonella* serovar *Typhimurium* and negative for other infectious agents. All animal protocols were reviewed and approved by the MSU Institutional Animal Care and Use Committee.

2.2. Flow cytometry and FACS

RBCs in whole blood were lysed in ACK (0.15 M ammonium chloride, 1 mM potassium carbonate, 0.1 mM EDTA disodium salt) buffer and lymphatic fluid cells were washed twice with PBS with 2% horse serum. A small volume of cells from blood and lymphatic fluid was stained with the following mouse anti-bovine antibodies (specificity): GD3.8 (pan γδ T cell), ILA29 (WC1, γδ T cell subset), CC21 (B cells), CC42 (CD2, αβ T cells and γδ T cell subset), BN180 (monocytes), BN115 (neutrophils), CACT116A (IL-2Rα; VMRD Inc., Pullman, WA). Flow cytometry was performed following standard protocols as previously described [9]. Because the changes in cell percentages between 0- and 6-h post-infection were highly variable, and were potentially residual fluctuations that occurred post-surgery, cell percentages at these two time points were averaged to arrive at a new time 0 post-infection value represented on the graphs.

For one mock infection (calf 156) and two experimental *Salmonella* serovar *Typhimurium* infections (calves 112 and 162), the lymphatic cells were stained with GD3.8 directly conjugated to FITC, washed, and sorted on a Vantage SE cell sorter (BD Immunocytometry Systems) as previously described [9]. Percent purities of the sorted cells were as follows: calf 112; 0 h αβ T cell 81%, 0 h γδ T cell 97%, 6 h αβ T cell 85%, 6 h γδ T cell 93%, 24 h αβ T cell 78%, 24 h γδ T cell 96%, 48 h αβ T cell 89%, 48 h γδ T cell 97%, 72 h αβ T cell 95%, 72 h γδ T cell 91%, calf 156; 0 h αβ T cell 98%, 0 h γδ T cell 89%, 48 h αβ T cell 82%, 48 h γδ T cell 89%, calf 162; 0 h αβ T cell 87%, 0 h γδ T cell 88%, 48 h αβ T cell 81%, 48 h γδ T cell 86%. The αβ T cell populations were contaminated by a mixture of cells, mainly B cells and a few γδ T cells, whereas γδ T cells were mainly contaminated with αβ T cells. Sorted γδ and αβ T cells were directly lysed in TRIzol reagent (Invitrogen; calf 112) or in Buffer RLT (Qiagen; calves 156 and 162) and genomic DNA sheared using Qiashredder columns, then frozen at −80 °C.

2.3. RNA extraction, amplification, and microarrays

RNA was extracted following the manufacturer's protocol for TRIzol (Invitrogen) extraction, or RNeasy (Qiagen) column purification, assessed on a Bioanalyzer 2100 (Agilent Technologies), and amplified either using Affymetrix Two-cycle (calf 112) target labeling protocol with 100 ng total RNA or the One-cycle protocol (calves 156 and 162) with approximately 1.6 μg of total RNA as described in the GeneChip® Expression Analysis Technical Manual (June 2004). Hybridizations to Genechip® Bovine Genome Arrays (Affymetrix) were performed with 15 μg biotin-labeled cRNA. Washing and staining was performed in the GeneChip® Fluidics Station 450 using the Midi_euk2v3 protocol. Chip scans were performed on the Affymetrix GeneChip® Scanner 3000. GeneChip® Operating Software (GCOS v.1.1, Affymetrix) [10,11] was used for data collection and analysis of gene lists using GCOS assigned detection calls and significance values of signal log ratios of >1 or <−1 (2-fold changes). Annotation of these lists was improved by approximately 50%