CD4+ T Cells Specific for \textit{C. difficile} Toxins are a Marker of Patients with Active Disease

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\textbf{Background:} Patients with IBD are at increased risk for infection by \textit{Clostridium difficile} (\textit{C. difficile}), a toxin-secreting bacteria that is the leading cause of nosocomial antibiotic-associated infectious diarrhea. One third (25-30\%) of patients with \textit{C. difficile} infection (CDI) relapse following antibiotic treatment, and IBD patients have a higher prevalence of relapse than non-IBD patients. The pathogenicity of CDI is known to require the activities of its toxins, TcdA and TcdB, but the T cell-mediated response to these toxins remains uncharacterized.

\textbf{Methods:} As a pilot study we collected blood from patients experiencing relapsing CDI, and from healthy volunteers with no history of CDI. CD4+ T cell responses to the toxins were measured using a flow cytometry assay that identifies antigen-specific CD4+ T cells by co-expression of CD25 and OX40 following 44h incubation with antigen. To define whether CDI infection polarized CD4+ T cells into specific Th cell subsets, expression of CCR4, CXCR3, CCR6, and CD39 was measured on antigen-specific cells.

\textbf{Results:} T cell responses to TcdB were significantly higher in patients than controls (median 1.01\% vs. 0.28\%; \(p = 0.041\)) and were significantly higher than patient TcdA responses (median 1.01\% vs. 0.10\%; \(p < 0.001\)). Positive control (Pediaceel) and TcdA T cell responses were not significantly different between patients and controls. TcdB-specific CD4+ T cells were functionally heterogeneous, on average: 25\% expressed the gut homing marker integrin \(\beta7\); there was a 1:1 ratio of Tregs to T effectors; and T effectors contained Th1, Th2 and Th17 cells at a 1.5:1:3 ratio. Anti-TcdA/TcdB IgG antibody titres were not significantly different between patients and controls.

\textbf{Conclusions:} Our data indicate that anti-TcdB CD4+ T cell responses are a more specific marker of disease than antibody titres. We are now investigating whether these responses can be used to predict relapsing CDI in IBD patients. Understanding the mechanistic basis for why IBD patients are more susceptible to CDI has the potential to inform the development of vaccines, to predict relapse and potentially uncover underlying immune mechanisms of dysbiosis in IBD.