BACTERIA SPECIFIC IL-10 SECRETING T-CELLS DERIVED FROM THE GUT ARE CROSS-REACTIVE WITH TUMOR ANTIGENS AND ACCELERATE TUMOR GROWTH IN MOUSE MODELS

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Background
We developed a method of CD4 epitope identification that includes selecting Class II interacting sequences via a multi-algorithm followed by functional phenotyping. We have evaluated 152 epitopes from 17 non-mutated tumor antigens (TA) and demonstrated Class II restricted epitopes could be identified which elicit either a selective Type I (IFN-gamma) or Type II (IL-10) response across multiple human PBMC (n=40). IL-10 inducing TA epitopes often shared a >50% identity and cross reactivity with multiple gut bacterial species. We questioned how prevalent these bacteria-tumor antigen (BAC-TA) cross reactive T-cells were in humans and whether these cells had any effects in cancer.

Methods
IL-10 ELISPOT quantified BAC-TA T-cells in human PBMC, murine spleen, and tumor infiltrating lymphocytes (TIL). Human and murine T-cell lines were tested against bacteria, TA, and controls to show cross reactivity and specificity. Murine BAC-TA T-cells were used for adoptive transfer. T-cells were characterized by cytokine array, PCR, and flow cytometry. The C3(1)-Tag transgenic model of mammary cancer was used to assess effects of BAC-TA T-cells on tumor growth.

Results
Measurable BAC-TA T-cells occurred in up to 90% of PBMC. TA epitopes with the highest incidence of response shared significant sequence homologies with greater than 10 bacterial species. TA specific T-cell lines from multiple donors showed significant reactivity to homologous bacteria and recombinant TA protein, but not unrelated bacteria and protein. Human BAC-TA T-cells secreted IL-6 and IL-10, were memory T-cells, and expressed genes similar to intraepithelial lymphocytes. Similar BAC-TA T-cells were identified in mice. P. aeruginosa-specific T-cells generated from FVB mice secreted significantly more IL-10 when stimulated with the 70% homologous peptide YB1-p82-96 as compared to HIV peptide; p=0.0004. We implanted a syngeneic tumor cell line into C3(1)-Tag. After tumor was established, fluorescently-labeled P. aeruginosa-YB1 specific T-cells were injected. Significantly increased florescence was seen in 100% of tumors and no fluorescence in mice injected with labeled naïve splenocytes (p<0.0001). Tumor volume 20 days after transfer was increased 55% in mice receiving P. aeruginosa/YB1-T-cells compared to splenocytes (p<0.0001). When C3(1)-Tag developed spontaneous tumors (500±75 mm³) TIL analysis revealed numerous IL-10-secreting BAC-TA T-cells that had migrated from blood to tumor.

Conclusions
A select group of bacteria are associated with the majority of homologies driving generation of BAC-TA T-cells. We have also identified bacteria never associated with TA homologies. These data lay the foundation for precision probiotics designed to reduce the BAC-TA memory T-cell pool.

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Ethics Approval
University of Washington Institutional Approval was obtained for all animal work shown here (#2878-01). All blood samples were obtained with written informed consent by the University of Washington Human Subjects Division (Protocol #7721).