Association of *Bacteroides fragilis* and enterotoxigenic *Bacteroides fragilis* with specific T-cell subsets in the colorectal carcinoma microenvironment

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BACKGROUND

- Experimental studies showed that enterotoxigenic *Bacteroides fragilis* (ETBF), in particular strains of *Bacteroides fragilis* (BF), could contribute to colorectal tumorigenesis through immune-mediated inflammation.
- However, the interactive effect of BT and ETBF, and T cell infiltrates in colorectal cancer (CRC) is still unclear.

HYPOTHESIS

T cell infiltrates might differ by BF and ETBF abundance in human CRC tissue

METHODS

- **Study design:** Cross-sectional study.
- **Study population:** We included 827 CRC cases with available BF, ETBF, and T cell data, that occurred in the Nurses' Health Study (121,701 women aged 30-55 years followed since 1976) and the Health Professionals Follow-up Study (51,529 men aged 40-75 years followed since 1986). Archival formalin-fixed paraffin-embedded tumor tissue blocks of confirmed CRC cases were collected.
- **Assessment of T cell density**: T cell subset density in intraepithelial and stromal regions was assessed by a customized 9-plex multispectral immunofluorescence assay (CD3, CD4, CD8, CD45RA, CD45RO, FOXP3, KRT, MKI67, and DAPI) with digital image analyses and pathologists' supervised machine learning algorithms.
- **Quantification of tumor BF and ETBF abundance:** The amount of BF and ETBF DNA was measured by a quantitative PCR assay.
- **Statistical analysis**: Spearman correlation test was used to assess correlations between the abundance of BF / ETBF and T cell densities. We also used multivariable-adjusted logistic regression models to assess the associations after adjusting for potential confounding.

CONCLUSIONS

- **High abundance of *Bacteroides fragilis* was associated with a lower stromal $CD3^+CD4^+$ regulatory T cell density in the CRC microenvironment.**
- **Our findings support the interactive effect of *Bacteroides fragilis* on specific T cells, which could explain why colorectal tumors harbor highly variable *Bacteroides fragilis* abundances and T cell densities.**

RESULTS

Table 1. Correlation between BF/ETBF DNA amount in tumor tissue and density of T cell subsets in tumor intraepithelial and stromal regions[1]

	Bacteroides Fragilis		Enterotoxigenc *Bacteroides Fragilis*	
T cell subset	rho	p-value	rho	p-value
Intraepithelial region				
$CD3^+CD4^+$ naïve	-0.0083	0.81	0.024	0.49
$CD3^+CD4^+$ memory	-0.031	0.37	0.020	0.57
$CD3^+CD4^+$ regulatory	-0.039	0.26	0.0041	0.91
$CD3^+CD8^+$ naïve	-0.018	0.61	0.014	0.69
$CD3^+CD8^+$ memory	-0.025	0.47	-0.0010	0.98
$CD3^+CD8^+$ regulatory	-0.047	0.18	0.0044	0.90
$CD3^+CD4^-CD8^-$ naïve	0.0029	0.93	0.082	0.019
$CD3^+CD4^-CD8^-$ memory	-0.027	0.44	0.015	0.67
Stromal region				
$CD3^+CD4^+$ naïve	-0.072	0.037	-0.047	0.18
$CD3^+CD4^+$ memory	-0.081	0.019	-0.011	0.75
$CD3^+CD4^+$ regulatory	-0.11	0.002	-0.059	0.092
$CD3^+CD8^+$ naïve	-0.029	0.40	-0.019	0.59
$CD3^+CD8^+$ memory	-0.024	0.49	0.024	0.49
$CD3^+CD8^+$ regulatory	-0.044	0.21	-0.022	0.52
$CD3^+CD4^-CD8^-$ naïve	-0.0058	0.87	0.045	0.20
$CD3^+CD4^-CD8^-$ memory	-0.031	0.37	-0.011	0.76

Table 2. Ordinal logistic regression analysis to assess the associations of BF/ETBF abundance (predictor) with T cell density (outcome)

	Univariable OR (95%CI)	Multivariable OR (95%CI)[1]
$CD3^+CD4^+$ naïve T cell density		
Amount of *Bacteroides fragilis*		
Negative	1 (referent)	1 (referent)
Low	1.02 (0.76-1.38)	1.01 (0.75-1.37)
High	0.77 (0.57-1.05)	0.75 (0.56-1.02)
P trend[2]	0.13	0.095
$CD3^+CD4^+$ memory T cell density		
Amount of *Bacteroides fragilis*		
Negative	1 (referent)	1 (referent)
Low	0.97 (0.72-1.31)	0.94 (0.70-1.27)
High	0.82 (0.61-1.11)	0.82 (0.60-1.11)
P trend[2]	0.22	0.16
$CD3^+CD4^+$ regulatory T cell density		
Amount of *Bacteroides fragilis*		
Negative	1 (referent)	1 (referent)
Low	0.88 (0.65-1.20)	0.87 (0.63-1.19)
High	0.68 (0.50-0.93)	0.61 (0.45-0.84)
P trend[2]	0.019	0.0034

1. Spearman correlation test was used.
2. Multivariable ordinal logistic regression model initially included age, sex, year of diagnosis, family history of colorectal cancer, tumor location, microsatellite instability, CpG island methylator phenotype, long-interspersed nucleotide element-1 methylation level, and *KRAS*, *BRAF*, and *PIK3CA* mutation status. A backward elimination with a threshold P of 0.05 was used to select variables for the final model.
3. P trend was calculated by the linear trend across the ordinal categories of *Bacteroides fragilis* DNA amount (negative, low, and high, as an ordinal predictor variable).