Oral therapy with colonization factor antigen I prevents development of type 1 diabetes in Non-obese Diabetic mice

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Supplemental Table 1. Antibodies used for Flow Cytometry in LL-CFA/I Treg Studies.

| Antibody specificity | Clone     | Source   | Dilution |
|----------------------|-----------|----------|----------|
| CD19                 | EBio1D3   | eBioscience | 1/600    |
| CD25                 | PC61.5    | Biolegend | 1/500    |
| CD39                 | 24DMS1    | eBioscience | 1/500    |
| CD4                  | RM4-5     | Biolegend | 1/500    |
| CD49B                | HMa2      | BD       | 1/400    |
| CD8α                 | 53-6.7    | eBioscience | 1/500    |
| CTLA-4               | UC10-4F10-11 | BD     | 1/500    |
| Foxp3                | FJK-16s   | eBioscience | 1/250    |
| IFN-γ                | XMG1.2    | BD       | 1/250    |
| IL-10                | JES5-16E3 | eBioscience | 1/250    |
| Lag-3                | C9B7W     | Biolegend | 1/400    |
| PD-1                 | RMPI-30   | eBioscience | 1/500    |
| Tbet                 | 4B10      | Biolegend | 1/250    |
| TCR-β                | H57-597   | Biolegend | 1/500    |
| TGF-β (Lap)          | TW7-16B4  | Biolegend | 1/400    |
| TIGIT                | 1G9       | Biolegend | 1/400    |
| TNF-α                | MP6-XT22  | Biolegend | 1/250    |

Summary of specificity, clone, and source of antibodies used in flow cytometry analysis.
**Supplemental Table 2.** Primer sequences for detection of cytokine-specific mRNA.

| Gene Name | Primer | Size | Sequence (5'→3') |
|-----------|--------|------|------------------|
| IL-6      | F      | 23   | TAGTCCTTCTACCCCAATTTC |  
|           | R      | 21   | TTGGTCCTTGGCCTCTTC  |
| TNF-α     | F      | 23   | CCCTCACACTCATCATCTCT |  
|           | R      | 19   | GCTACGACGTGGGCTACAG  |
| IL-10     | F      | 21   | GCTCTTACTGACTGACGAG  |  
|           | R      | 20   | CGCAGCTCTAGGAGCATGTG  |
| IL-33     | F      | 21   | ACAGATATATGACTTACGGCG |  
|           | R      | 23   | AAATGGACCCCTCTAAGCAAA |
| GAPDH     | F      | 20   | ACCACAGTCCATGCCATCAC |  
|           | R      | 19   | TCCACCACCGGCTGTGCTGA  |
| β-actin   | F      | 22   | ATCTACGAGGCTATGCTCTCC |  
|           | R      | 21   | AGCCTCGGTCAGATCTCAT  |
Supplemental Figure 1. Negative controls for tetramer staining. Four wk-old NOD females were orally dosed with $5 \times 10^7$ CFUs of LL-CFA/I, LL vector, or PBS (n=5/group). Additional doses were given every 2 wks. At 11 wks of age, lymphocytes from the PaLNs were isolated and labeled with tetramers specific for (A) human CLIP or (B) TUM peptide.
Supplemental Figure 2. LL-CFA/I does not Induce Negative Regulators in NOD Mice

Four wk-old NOD females were orally dosed with 5x10^7 CFUs of LL-CFA/I, LL vector, or PBS (n=5/group). Additional doses were given every 2 wks. At 11 wks of age, Foxp3^+CD4^+ Tregs from the (A) spleens and (B) MLNs were examined for expression of PD-1, CTLA-4 and Tigit.

Supplemental Figure 2. LL-CFA/I does not induce negative regulators in NOD mice. Four wk-old NOD females were orally dosed with 5x10^7 CFUs of LL-CFA/I, LL vector, or PBS (n=5/group). Additional doses were given every 2 wks. At 11 wks of age, Foxp3^+CD4^+ Tregs from the (A) spleens and (B) MLNs were examined for expression of PD-1, CTLA-4 and Tigit.
Supplemental Figure 3. LL-CFA/I promotes regulatory environment in the gut. Eight week old BALB/c mice (n=9-14/group) were orally dosed with $2 \times 10^9$ CFUs of LL-CFA/I, LL-vector or PBS. After 1.5 hours, small intestines and Peyer’s Patches were collected. Expression of cytokines was analyzed as fold change against the PBS control group. *$p<0.05$, **$p<0.005$, and ***$p<0.0001$ for LL-CFA/I vs LL-vector.
Supplemental Figure 4. Mouse Microbiota are not Significantly Different Between Groups Before LL Treatments Begin. (A) Pair-wise comparisons of beta diversity post-treatment (11 weeks old). (B) Alpha diversity of pre-treatment samples at 4 weeks old. (C) PCoA plots of beta diversity in pre-treatment samples. NS, not significant.
Supplemental Figure 5. LL-CFA/I does not Induce Splenic IFN-γ⁺IL-10⁺ Foxp3⁺CD4⁺ Tr1 cells at 17 weeks

Supplemental Figure 5. LL-CFA/I does not Induce Splenic IFN-γ⁺IL-10⁺ Foxp3⁺CD4⁺ Tr1 cells at 17 weeks. Four wk-old NOD females were orally dosed with 5x10⁷ CFUs of LL-CFA/I, LL vector, or PBS (n=5/group). Additional doses were given every 2 wks from 6 to 16 weeks of age. (A) Splenocytes were stimulated with anti-CD3 and anti-CD28 mAbs and CD25⁺Foxp3⁺CD4⁺Tregs analyzed for expression of IFN-γ and IL-10.