Supplemental Digital Contents

SDC, Materials and Methods

Immunofluorescent Staining

After the sections (5 μm) were fixed and blocked, the primary antibodies against CD4, CD8 and CD45 (Abcam, Cambridge, UK) were incubated overnight at 4°C. Sections were incubated with Alexa Fluor 633-conjugated secondary antibodies, stained with DAPI (4’, 6-diamidino-2-phenylindole). The slides were visualized using a confocal scanning microscope (Leica TCS SP2, Heidelberg GmbH, Mannheim, Germany). The numbers of positive cells were quantified by NIH Image-Pro Plus 6.0 software.

Isolation of Mucosal Lymphocytes

IEL and LPL were isolated as described previously (1). Briefly, intestinal segments (10 cm) were cut into pieces and incubated in Hanks’ solution with 0.75 mM EDTA. The supernatant was passed through 80 and 400 grit screen gauze to remove debris. The filtered cells were centrifuged through a 35%/60% Percoll gradient to collect IEL. The remaining pieces were used for isolation of LPLs. The viability of lymphocytes was determined >90% by trypan blue exclusion. The lymphocyte count was measured by a hemocytometer.

Flow Cytometric Analysis

Cells were stained with fluorescently labeled antibodies: CD3-allophycocyanin (BD Pharmingen, San Diego, CA), CD4-fluorescein isothiocyanate, CD8-phycoerythrin (PE) or TCR γδ-PE (eBioscience, San Diego, CA). Isotype-matched antibodies were used as negative controls. The cells were run on a FACSCalibur cytometer (BD Biosciences, San Jose, CA), and 10,000 events were analyzed using CellQuest software (BD Biosciences). The absolute numbers of
specific cell populations were calculated by multiplying the respective percentages by the whole number of lymphocytes. Relative proportions of circulating Treg cells were analyzed using FITC-labeled CD4, PerCP-labeled CD25 and PE-labeled FoxP3 antibodies. Intracellular staining for FoxP3 was performed according to the manufacturer’s protocol (eBioscience).

**Denaturing Gradient Gel Electrophoresis (DGGE) and Sequencing Analysis**

The DNA of fecal and mucosal samples was extracted using the QIAamp DNA Mini Kits (Qiagen, Valencia, CA). The fragments of 18S rRNA gene were amplified with the primer sets NS1/FR1 and EF390/GC-FR1 (2). The amplicons were separated on a D-Code universal mutation detection system (Bio-Rad) (2). DGGE profiles were analyzed using QuantityOne software 4.2 (Bio-Rad). Similarities between samples were determined based on the Dice similarity coefficient and the unweighted-pair group method. Species richness and community diversity were evaluated as DGGE band numbers and Shannon-Wiener diversity indices (3). The intensity of each band was expressed as a proportion (%) of the sum of all defined bands in the same lane (4). The quantitative information derived from relative band quantities per band type per sample was exported as a data matrix, which was subjected to the redundancy discrimination analysis (RDA). In this analysis, relative intensity of each band was considered as species variable, and the immunological parameters as environmental variables. The significance and proportion of the total variance in community structure were detected with the Monte Carlo permutation test using CANOCO 4.5 software package (Microcomputer Power, Ithaca, NY). The predominant bands were sequenced to gain the closest known relatives using the BLAST programs in GenBank (2). The phylogenetic trees were constructed by neighbour joining using MEGA 4 software (5).

**Assessment of Serum Cytokines**
The enzyme-linked immunosorbent assay kits for monkey IFN-γ (Mabtech Inc., OH) and IL-10 (Abcam PLC, Cambridge, UK) were used to determine serum concentration of the cytokines.

**Statistical Analysis**

All data were expressed as means ± standard deviations (SD). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Holme-Sidak test using the SPSS software (version 12.0). For detection of correlation we performed linear regression analysis using Pearson test. A P value of less than 0.05 was considered significant.

**Reference**


SDC, **Figure S1.** The depletion and repopulation in peripheral blood lymphocytes (CD4⁺, CD8⁺, and CD3⁺ T cells) after alemtuzumab treatment in cynomolgus monkeys. (A) The values in treated groups are expressed as the percent of cells remaining after treatment relative to the number of cells in control monkeys (%). (B) Variations of cell counts in peripheral blood. Results shown are the mean ± SD (n = 3), *P<0.05, **P<0.01, compared with the controls.
SDC, Figure S2. Immunofluorescent analyses of immune cells in colon of cynomolgus monkeys by alemtuzumab treatment. Longitudinal visualization of depletion and repopulation of CD4⁺, CD8⁺ and CD45⁺ cells (red) in the control and treated animals were performed by confocal laser scanning microscopy. Nuclei were stained with DAPI (blue). (A) Representative images of immunofluorescent staining. (B) The numbers of CD4⁺, CD8⁺, and CD45⁺ cells per HPF (magnification, ×200) in the colon were measured by Image-Pro Plus 6.0. Data are presented as mean ± SD (n = 3), * P<0.05, **P<0.01, compared with the controls.
**SDC, Figure S3.** Phylogenetic tree generated from the partial 18S rDNA sequences.

The sequences were aligned with closely related 18S rDNA sequences retrieved from GenBank database using the BLAST. The tree was conducted by the sequences of the predominant fungal species in the feces through the MEGA software. The scale bar represents the genetic distance.
**SDC, Figure S4.** Phylogenetic relationship of 18S rDNA sequences obtained from the mucosa-associated communities. The tree was generated from the sequences of the predominant fungal species in the colonal mucosa. The scale bar represents the genetic distance.
SDC, Figure S5. Comparison of the fungal communities in the ileal mucosa after alemtuzumab treatment. (A) Clustering analysis and representative fingerprinting of the mucosa-associated fungal populations in the ileum. The scale bar indicates similarity (%). I01 to I11 indicate the prominent bands. (B) The comparison of fungal community diversity. Values are expressed as mean ± SD (n = 3). Shifts of the relative abundance in the predominant fungal phylotypes at the class (C) and species levels (D).
**SDC, Figure S6.** Phylogenetic tree of the partial 18S rDNA sequences identified in the DGGE profiles. The phylogenetic tree was built from sequence data of the DGGE profiles from the ileal mucosa. The scale bar represents the genetic distance.
SDC, Figure S7. Redundancy discrimination analysis (RDA) showing the correlation of colonic mucosal fungal community with lymphocytes and cytokines. Direction of arrow indicates the parameters associated with changes in the community structure, and the length of the arrow indicates the magnitude of the association. The percentage of variation explained by Axis 1 and 2 is shown in brackets.