probe. After post-hybridization washes, nonspecific binding of antibodies was blocked by immersing the sections in 5% nonfat milk in phosphate-buffered saline (PBS) for 30 min. Sections were then treated with a 1:300 dilution of CD68 mAb (Dako, Carpinteria, CA) at 4°C overnight. After washing, slides were developed with a peroxidase-conjugated secondary antibody and diaminobenzidene substrate according the manufacturer's protocol (ABC Elite kit; Vector Labs, Burlingame, CA). The sections were then washed in PBS and 0.1× PBS, dehydrated in ethanol containing 0.3 M ammonium acetate, and coated with nuclear track emulsion. After development, the slides were briefly counterstained in hematoxylin.

18. Longer autoradiographic exposures of 7 to 10 days increase the sensitivity of in situ hybridization. To determine the limits of detection of HIV RNA, we first measured the background signal over MNCs and FDCs from the binding of antisense HIV RNA probe to LT sections of HIV-seronegative individuals and from the nonspecific binding of the sense HIV RNA probe to LT sections of HIV-seropositive individuals. Dividing the number of silver grains determined by quantitative image analysis in randomly selected areas by the number of cells in those areas provided a maximum estimate of background. Backgrounds by both approaches were one or two silver grains per MNC for 10-day exposures. An MNC with one HIV RNA copy will show 24 grains over background with such long exposures and will be easily identified. The lower limit of detection for MNCs is therefore about one copy of HIV RNA per cell. For the FDCs, we measured the area of germinal centers (GCs) and determined their average backgrounds to be 2.3 grains per 10^{-3} mm^2. The Poisson probability that x number of grains differs from a background average of m grains is $1 - m^x \exp(-m)\, m(x!)^{-1}$ (*22*). For a probability $P > 0.99$ that there is a significant increase over background in a GC, the signal over FDCs would have to be more than seven grains per 10^{-3} mm^2. For example, the GC shown in Fig. 4B had 450 grains in an area of 0.05 mm^2, or 9 grains per 10^{-3} mm^2, and thus fulfills this condition; from the number of silver grains over background, we calculate that there are 146 copies of HIV-1 RNA in this GC. The limits of detection of viral RNA for infected cells expressed per gram of tissue depends on the number and area of the sections examined. For example, we usually randomly sampled and exhaustively screened 40 8-μm sections averaging ~5 mm^2 each, cumulatively equivalent to ~1.2 mg of tissue. If these sections contained no MNCs with HIV-1 RNA, this frequency would be <~625 cells per gram. For the FDCs, we determined with the long exposure an average detectable copy number of ~20 copies per GC and thus a lower detection threshold of ~10^4 copies per gram of LT.

19. For DNA PCR analysis, nucleic acids from guanidine-HCl extracts of flash-frozen tonsil biopsies were precipitated out of 70% ethanol, pelleted, ethanol-washed, and resuspended in 10 mM tris-HCl (pH 8.0) containing 1 mM EDTA and 0.5% SDS. The suspensions were then treated with ribonuclease A (RNase A, 20 μg/ml) for 1 hour at 37°C, incubated with proteinase K (100 μg/ml) at 50°C for 3 hours, and finally extracted with phenol, phenol/chloroform, and chloroform before ethanol precipitation and wash. Control DNA, with and without HIV-1 proviral DNA, was similarly extracted from ACH-2 cells and H-9 cells, respectively. Nested PCR was performed with the use of two sets of primers designed to amplify a conserved region of HIV-1 *gag*. The primary amplification reactions contained 1 μg of each DNA specimen, 1× PCRII reaction buffer (Perkin-Elmer), 2.5 mM $MgCl_2$, 200 mM deoxynucleotide triphosphates, 1 μM primers (30-nucleotide oligomers: 5′-GTCAGCCAAAATTACCCTATAGTGCAGAAC-3′, 5′-ACATAGTCTCTAAAGGGTTCCTTTGGTCCT-3′), and Amplitaq polymerase (0.08 U/ml, Perkin-Elmer) in a 100-ml volume. DNA was amplified 25 cycles at 94°C for 45 s, 60°C for 45 s, and 72°C for 3 min. The secondary (nested) reactions contained 2 μl of the primary amplified reaction mixture with otherwise the same constituents, except for primers (20-nucleotide oligomers: 5′-TCACCTAGAACTTTAAATGC-3′, 5′-ATTTAATCCCAGGATTATCC-3′). This second amplification was 30 cycles with the same cycling parameters. RT-PCR reactions were performed on RNA containing fractions of the guanidine tissue homogenates and were processed and PCR-amplified in the same manner, except that deoxyribonuclease pretreatment was substituted for RNase A pretreatment and reverse transcription using rTth polymerase (Perkin-Elmer) was included before amplification. Southern (DNA) blots were hybridized to a cocktail containing three oligonucleotide probes labeled at the 3′ termini with [^{32}P]deoxyadenosine triphosphate specific for sequences internal to the nesting primers (30-nucleotide oligomers: 5′-CATGGGTAAAAGTAGTAGAAGAGAAGGCTT-3′, 5′-GGGACATCAAGCAGCCATGCAAATGTTAAA-3′,5′-CCAAGGGGAAGTGACATAGCAGGAACTACT-3′).

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TECHNICAL COMMENTS

Interactions Between Epithelial Cells and Bacteria, Normal and Pathogenic

Lynn Bry *et al.* show that the monoassociation of germ-free (GF) mice with wild-type *Bacteroides thetaiotaomicron* induced expression of an α1,2 fucosyltransferase messenger RNA and production of fucosylated glycoconjugates that were reactive with *Ulex europaeus* agglutinin I in the epithelial cells of the small intestine (*1*). A mutant mouse strain that lacks the ability to utilize L-fucose did not induce efficient epithelial fusion. We have also observed the induction of an α1,2 fucosyltransferase that mediates the synthesis of the fucosyl asialoGM1 glycolipid of small intestinal epithelial cells during the first stage of microbial colonization (conventionalization) in GF mice (*2*). Recently, we found that this fucosylation was induced by an indigenous bacteria [segmented filamentous bacteria (SFB) (*3*), which was identified on the basis of its 16*S* ribosomal DNA sequence (*4*)] and that it resulted in expression of major histocompatability complex class II (MHC II) molecules, expansion of intraepithelial lymphocytes (IEL), and increase in immunoglobulin A (IgA)–producing cells. Within a month after SFB colonization, the columnar cell-to-goblet cell ratio and the mitotic activity of cryptal cells were almost the same as those found in wild-type mice. We have also found that when the SFB colonization in the conventionalization process was selectively inhibited by the oral administration of a monoclonal antibody against SFB, MHC II expression, and the growth of αβ–T cell receptor–bearing IELs and IgA-producing cells were repressed (*5*). Thus, SFB seem to be essential for altering or accelerating the development of the small intestine. These events should occur in the weaning stage in the case of conventional mice with a normal intestinal microflora.

Alteration of the developmental program did not occur in the course of association of GF mice with indigenous microbes derived from rat or human feces (*6*). SFB derived from mice and rats did not cross-colonize in rats and mice, respectively (*7*). There appears to be a strict limit to the interaction between the host animal and the intestinal bacteria, in accord with the concept of "autochtonous bacteria" proposed by Dubos *et al.* more than 30 years ago (*8*). Does the association of GF mice with *B. thetaiotaomicron* induce class II expression, expansion of IEL and IgA-producing cells, and so on after the expression of an α1,2 fucosyltransferse? What is the original host of this bacterium, mouse or human? A GDP-fucose:asialo GM1 α1,2 fucosyltransferase was induced in GF mice on injury to the small intestine (*9*). In our study, α1,2 fucosyltransferase induction was the first event. We have no evidence, however, to suggest that this fucosylation initiates the developmental program of the intestinal mucosa, including the components in the lamina propria.

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Response: Development of the mouse small intestine is often viewed in terms of the cytodifferentiation of its endoderm that occurs in late fetal life, or the formation of its crypt-villus units, which is completed during the first three postnatal weeks. Umesaki *et al.* emphasize the importance of having a broader vision of gut development. We agree. A "trialogue" between the intestinal microbiota, the self-renewing intestinal epithelium, and the diffuse gut-associated lymphoid tissue (GALT) is probably critical in forming and maintaining this dynamic ecosystem. Studies by Umesaki *et al.* provide strong evidence that a component of the normal microbiota can influence the composition of the diffuse GALT. Colonization with the *B. thetaiotaomicron*–type strain, VPI-5482, is associated with similar composition changes. For example, an influx of IgA^+ B cells occurs after exposure to this organism.

One should consider the diffuse GALT's composition, but also its spatial complexity, which has been hard to characterize because markers are difficult to detect with conventional immunohistochemical methods. More sensitive techniques (*1*) have allowed us to examine these features in mice that contain a normal (conventional) microbiota. For example, αβ T cells populate the intraepithelial and lamina propria compartments in crypts and villi, while γδ T cells are limited to the villus epithelium. T_H1 and T_H2 cells appear predominantly in the lamina propria of the villus (*2*).

Like the diffuse GALT, components of the microbiota are arranged asymmetrically along crypt-villus units: SFB attach to epithelial cells located in the upper two-thirds of the villus. Histochemical stains of unperfused small intestines obtained from specified pathogen-free conventional mice suggest that crypts are not colonized by this or other bacterial species. The asymmetric distribution of the microbiota may serve to organize components of the diffuse GALT. Conversely, the diffuse GALT may influence the spatial organization of the microbiota.

The diffuse GALT also communicates with the intestinal epithelium: Mice that lack γδ T cells have fewer crypt epithelial cells and slower epithelial cell migration up the villus (*3*). Contaminating adult GF mice with SFB or VPI-5482 reveal another component of this trialogue: communication between the microbiota and the gut epithelium. VPI-5482, which was originally recovered from a human, signals the epithelium to induce and sustain α1,2 fucosyltransferase gene transcription and production of fucosylated glycoproteins and glycolipids. This is not a nonspecific response of the epithelium to bacterial colonization. Monocontamination of GF NMRI mice with two other anaerobes that normally colonize the mouse and human intestine, *Peptostreptococcus micros* and *Bifidobacterium infantis*, produces no detectable effect on fucosylated glycoconjugate production (*4*).

Unlike SFB, signaling occurs without direct bacterial attachment to enterocytes (*5*). Signaling depends on the ability of the organism to use fucose as a carbon source (*5*). We recently found that the *B. thetaiotaomicron* genome contains a locus analogous to the *Escherichia coli* fucose utilization regulon (*6*). A Tn4351 insertion renders the Fu-4 strain of *B. thetaiotaomicron* unable to use fucose and unable to signal enterocytes to produce fucosylated glycoconjugates. The site of insertion is the open reading frame of one of the genes within this locus (*7*). Monocontamination of GF mice with isogenic strains of *B. thetaiotaomicron* that contain engineered disruptions of each gene in the regulon should provide clues about the nature of the signal that emanates from this metabolic pathway.

To induce and sustain fucosylated glycoconjugate production in enterocytes, VPI-5482 must reach a critical population density (*5*). This requirement may reflect secretion of a soluble bacterial factor that produces a concentration-dependent response in the epithelium. Or there may be a density-dependent change in the metabolic properties of the bacteria that affects production of a signaling molecule—a process known as "quorum sensing" (*8*). In the mammalian gut, where there is a highly complex society of microorganisms, secreted signaling molecules may allow communication between (and within) bacterial species. Multiple species may cooperate to generate a concerted signal that establishes a mutually beneficial niche. Such density-dependent signaling systems may also interfere with one another if a similar set of molecules is used by different species to modulate distinct metabolic pathways. This type of interference could allow the microbiota to prevent the encroachment of pathogens. If such encroachment occurs, the response of the host may depend on the relative locations of the pathogen, components of the diffuse GALT, and members of various intestinal epithelial lineages—factors that likely are influenced by the trialogue.

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Determining the Early History of El Niño

Daniel H. Sandweiss *et al.* (*1*) reiterate arguments advanced a decade ago (*2*) that climatic and oceanic changes 5000 years before present (B.P.) resulted in the onset of El Niño/Southern Oscillation (ENSO) events along the coast of Peru. A major argument used to support this conclusion is the occurrence of southwardly displaced tropical molluscan assemblages in natural deposits and shell middens older than 5000 years B.P. along the coast of northern Peru. One of the best examples of such a thermally anomalous molluscan assemblage (TAMA) is found in the paleo-lagoon at Santa (9°S). A detailed geological and paleoecological study (*4*) showed that the presence of the Santa TAMA was the result of changes in coastal morphology, not climate. Contrary to the contention of Sandweiss *et al.* (*1*, p. 1532, and notes 22 and 23), DeVries and Wells (*4*) showed that the Santa TAMA developed in a warm, narrow embayment open to the ocean and coexisted with temperate species then occupying