probes. After post-hybridization washes, nonspecific binding of antibodies was blocked by immersing the sections in 5% nonfat milk in phosphate-buffered saline (PBS) for 1 hour. Immunostained sections were then treated with 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 visualized with diaminobenzidine as substrate according to the manufacturer's protocol (ABC Elite kit; Vector Labs, Burlingame, CA). The sections were then washed in PBS and 1:10 PBS, dehydrated in ethan- 
ol containing 0.3 M ammonium acetate, and coated with nuclear track emulsion. After development, the slides were briefly counterstained in hematoxylin.

Longer autoradiographic exposures of 7 to 10 days increased 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 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 mm2. The Poisson probability that x grains is expected from a background average of m grains is 1 – $e^{-m} (m/x)^x$ (22). For a probability $P > 0.99$ that there is a significant increase in silver grains in a GC, the signal over FDCs would have to be more than seven grains per 10–3 mm2. For example, the GC shown in Fig. 4B had 450 grains in an area of 0.05 mm2, or 9 grains per 10–3 mm2, 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 infect- ed 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 mm2 each, cumulatively equivalent to ~1.2 mg of tissue. The second set contained no MNCs, and the background over HIV-1 RNA, this frequency would be ~625 cells per gram. For the FDCs, we determined with the long exposure of the detectable copy number of ~20 copies per GC and thus a lower detection threshold of ~105 copies per gram of LT.

19. For DNA PCR analysis, nucleic acids from guanidine- 
HCl extracts of flash-frozen tissue biopsies were pre- 
cipitated out of 70% ethanol, pelleted, ethanol- 
ashed, and resuspended in 10 mM tris-HCl (pH 8.0) 
containing 1 mM EDTA and 0.5% SDS. The suspen- 
sions were then treated with ribonuclease A (RNase 
A, 20 μg/ml) for 1 hour at 37°C. Incubated with pro- 
teinase K (100 μg/ml) at 50°C for 3 hours, and finally 
extracted with phenol, phenol/chloroform, and chlo- 
roform-isooctyl alcohol (30-nucleotide oligomers: 3′- end oligonucleotides: 5′-TAACCACTAAGGATTTAAGGC-3′, 5′-CATGATGGCTTCTGCGAT-3′, 5′-GACATAGCCTTTGTCAC-3′), and Amplicon 
polymerase cycle primers (Perkin-Elmer) in a 100-μl 
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 am- plified reaction mixture with the same conditions, 
except for primers (20-nucleotide oligomers: 5′-TACCACTAAGGATTTAAGGC-3′, 5′-AT- 

**TECHNICAL COMMENTS**

**Interactions Between Epithelial Cells and Bacteria, Normal and Pathogenic**

Lynn Bry et al. show that the monoassocia- 
tion of germ-free (GF) mice with wild-type 
Bacteroides thetaiotaomicron induced expres- 
sion of an α1,2 fucosyltransferase messenger 
RNA and production of fucosylated glyco-
conjugates that were reactive with Ulex eu-
ropeanus 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 
testinal epithelial cells during the first 
stage of microbial colonization (convention-
alization) 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 16S ribosomal DNA se-
quene (4) and that it resulted in expression of 
major histocompatibility complex class II 
(MHC II) molecules, expansion of intraepi-
thelial 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 
mictotic activity of cryptal cells were almost 
the same as those found in wild-type mice. 
We have also found that 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 αT-β 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 
testine. 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 “au-
tochthonous bacteria” proposed by Dubos et 
als. more than 30 years ago (8). Does the 
association of GF mice with B. thetaiotaomi-
tron induce class II expression, expansion of 
IEL and IgA-producing cells, and so on after 
the expression of an α1,2 fucosyltransferase? 
What is the original host of this bacterium, 
mouse or human? A GDP-fucose:asialoGM1 
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 
testinal mucosa, including the components 
in the lamina propria.

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**REFERENCES AND NOTES**

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Response: Development of the mouse small intestine is often viewed in terms of the cytokindifferentiation 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) may influence the composition of the diffuse microbiota. The asymmetric distribution of the GALT may serve to organize components 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 α,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 F4 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 palaeoecological 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
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