AUTHOR COMMENTARIES - 2009

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David Relman

Featured Scientist Interview

A recent analysis of Essential Science Indicators℠ *from Thomson Reuters data showed that the work of Dr. David Relman had the highest percent increase in total citations in the field of Microbiology. His record in this field includes 24 papers cited a total of 1,467 times between January 1, 1999 and April 30, 2009.*

Dr. Relman is the Thomas M. and Joan C. Merigan Professor in the Department of Medicine, Division of Infectious Diseases and the Department of Microbiology & Immunology at Stanford University School of Medicine. He is also the Chief of Infectious Diseases for the VA Palo Alto Healthcare System.

In the interview below,* ScienceWatch.com *correspondent Gary Taubes talks with Dr. Relman about his highly cited research on human microbiota.

SW: As a pioneer of the modern study of human microflora, there must have been a moment when you first came on the subject and found it worthy of interest. How did that happen?

I was finishing a postdoctoral fellowship at Stanford with Stanley Falkow and my plan was to pursue a career in the research basis for microbial pathogenesis and combine that with some clinical care. The only catch was that I had gotten interested in what had started out to be a side project. It had less to do with the molecular basis of bacterial disease and more to do with pathogen discovery.

I was going to these weekly clinical case conferences, and a case was presented on a disease called bacillary angiomatosis, which was a relatively common problem in AIDS patients. The interesting feature was that it clearly seemed to be associated with a bacterial organism. When you looked at stains under the microscope you could see clusters of what were obviously bacteria in places you don't expect to find them. And yet no one had been able to identify these bacteria because they couldn't be cultivated. Because cultures are the mainstay of bacterial diagnosis, it meant there was no answer for this disease. Stan had been with me at the case conference, and afterward he said this ought to be solvable with molecular tools. He pointed me to the work of some leading environmental microbiologists who at the time—this was now the late 1980s—were looking at DNA sequences obtained directly from environments where there was obviously a great diversity of organisms. They were using these sequences to say what in the bacterial world might be there.

So I simply borrowed the approach of these environmental microbiologists and applied it to these clinical specimens and, lo and behold, a sequence emerged that we could associate with the presence of these visible organisms. It turned out to be a new sequence and an organism that to this day is believed to be the cause of bacillary

angiomatosis. It's also the cause of cat-scratch disease.

> *"...one of the most important ecosystems on the planet might be the human body."*

To me the important lesson was that it may be possible to identify previously unrecognized pathogens by taking this approach of trying to discover telltale diagnostic sequence information directly from clinical specimens. I started down that path as a parallel to what I was doing in bacterial pathogenesis, looking in different disease settings where there was a suspicion of a bacterial cause, yet nothing had been identified at that point. I started looking in different kinds of human tissue. The challenge was to find tissue that would potentially contain the pathogen, but wouldn't contain any of the myriad of normally occurring bacterial commensals, the bacteria that we live with all the time, in the human body.

SW: So the initial problem was how to identify the pathogen from the background of normal microflora?

That seemed to be the problem at the time. Using this molecular approach meant that, in theory, we would pick up any and all bacteria. We couldn't know upfront what the nature of the bacterial target or the pathogen might be. So we were targeting conserved sequences believed to be found in nearly all bacteria, which, of course, includes commensals. Along the way it became clear to me that what I was considering the background problem was actually an interesting topic unto itself.

SW: Who else was working on commensals at the time, and how were they approaching it?

Certainly this was nothing new in essence. Exploring commensal microbiota was a long-standing interest of many folks, but they were taking the more traditional approach—using cultivation and other kinds of classic microbiological approaches. I was interested in using the same kind of molecular survey tools that environmental microbiologists were using, so instead of finding one or a discrete number of sequences from the pathogen, I was finding hundreds or thousands of discrete sequences that would correspond to our normal indigenous microbiota. So for me, what became a new area of investigation was something that was previously, for me, a distraction and an annoyance. Using these molecular techniques, I started to deliberately sample the kinds of sites where I knew there would be a complex microbial community.

SW: Did you start with the human intestines, the subject of your highly cited 2005 *Science* article (Eckburg PB, *et al*., "Diversity of the human intestinal microbial flora," 308[5728]: 1635-8, 10 June 2005)?

The first deliberate study I did looking at indigenous microbiota was in the gum pocket, and it was actually my own gum pocket in this case. It was published in 1999 (Kroes I, Lepp PW, Relman DA, "Bacterial diversity within the human subgingival crevice," *Proc. Nat. Acad. Sci. USA* 96[25]: 14547-52, 7 December 1999). I had this idea as I was getting ready to go to my dentist. I went into work first, picked up some sterile collection tubes, brought them with me to the dentist's office, and asked him, as he was cleaning my teeth, would he mind putting this stuff into these tubes, instead of throwing it out.

Part of the idea was to compare this molecular approach to the classic microbiological culture-based approach on the same material. What we found was, not surprisingly, that the majority of organisms could be found only with the molecular approach, not with cultivation.

SW: What led you to move from the gum pocket to the intestine?

Well, it has been known for a while that the distal gut, the large intestine, is the site in the body most likely to contain the greatest density of bacteria. In the early 2000s, I was interested in the idea of comparing bacterial diversity in the setting of health vs. disease, as I still am. I was approached by a clinician in Canada, Charles Bernstein, who had collected an interesting set of colonic biopsy samples from patients with Crohn's disease. He's one of the co-authors on that 2005 *Science* paper. He said, "I have this population-based study. It's really well controlled; we have lots of clinical data, and we've also recruited healthy family members of patients with Crohn's disease. Would you be interested in studying these samples?" I said, "Sure." But I thought we should start by examining the healthy samples.

With Charles's help, we came up with a sampling scheme, going for defined regions of the large intestinal tract in each of the healthy controls, moving right down the large intestine, starting with the cecum. So we had these samples, and we also had the opportunity to sequence more deeply through a collaboration with some folks at what was then The Institute for Genomic Research and is now the J. Craig Venter Institute. They said that at low cost they could sequence clone libraries for us at much greater numbers than we could ever do on our own. So this seemed to be a good opportunity to re-examine the patterns of diversity in health from this well-studied site, but a site not well-studied with

these modern molecular techniques. That's how this study and paper came to be.

SW: Why do you think that paper has garnered so many citations in such a short time? What makes it so influential?

There are a couple of possible answers to that. It might be a chicken-and-egg or horse-and-cart kind of issue. First, the paper highlighted what seemed to be some pretty interesting features to this ecosystem that hadn't been appreciated or realized—in particular, the extent of the diversity in this microbiota and how that diversity varied from person to person and site to site. It suggested that one of the most important ecosystems on the planet might be the human body. All the interest in the microbial world was focused everywhere in the "outside" world, but less so within each of us, and what we learned was that this particular ecosystem was as interesting and diverse as any of them. By studying this ecosystem we might be learning about some fundamentally important aspects of human health and disease.

"...I was interested in the idea of comparing bacterial diversity in the setting of health vs. disease..."

The other point is that this paper may have just come along at the right time. It highlighted the value of bringing together capabilities and perspectives from different disciplines—clinical medicine, traditional ecology, environmental microbiology, etc.—at a time when people were just beginning to realize that this particular integration of fields might hold a lot of promise. I think our paper influenced funding agencies and other researchers to start thinking about focusing their efforts on this particular sampling site—the human body.

The fact that NIH and other institutions in the European Union have all decided to invest large amounts of money in what people are calling human microbiome projects has now, if for no other reason, drawn a lot more people into this area. Considering the economic challenges for the research community right now, to have this kind of money directed towards this topic has led to people setting aside what they were doing and turning to this area of investigation. At the time that this paper was published there were relatively few people deploying these kinds of perspectives and technologies on the human microbiota.

SW: How has research on human microflora evolved in the four years since you published that paper?

Well, in a couple of ways. First, an acceleration of science and technology forces and trends started to become important around 2005. One of the most obvious is the development of sequencing technologies, and the amazing, almost exponential growth in capabilities in DNA sequencing. Part of the attraction to that 2005 paper was just the number of sequences we were able to report at the time. Today, for the same amount of money and time, the number of sequences you can generate is astronomically larger.

As an example, we published a paper this past fall in *PLoS Biology* (Dethlefsen L, *et al*., "The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing," 6[11]: 2383-2400, November 2008), another look at microbial diversity in the distal gut in a study of the effect of antibiotics on a number of healthy individuals, and the number of sequences we were able to report was about 50-, almost 100-fold greater than in that 2005 paper, and for less money. So just the power of next-generation sequencing is one of the big changes and a dominant feature of the new scientific landscape.

The other way this research has evolved is in the increasing attraction of this field for people who were trained and then practiced in other fields of science. We now have some really prominent environmental microbiologists becoming interested in the human body. We have some very prominent microbial ecologists interested in the human body instead of grass lands or river sediment. We have some very gifted mathematicians and statisticians interested in data from the human body rather than data from economics or meteorology. It all creates a convergence of interest and insight and capability that's tremendously exciting.

SW: What would you say is your single favorite research project at the moment?

One thing I'm actively pursuing is the effect of deliberate perturbation of these microbial communities. The tool we're using is antibiotics. We still have a lot to learn about the nature of these communities and one way to do it is to study how they respond and behave when they're perturbed. This approach also has a lot of clinical relevance. Clinicians are interested in what antibiotics are actually doing, and we already know they have untoward effects. Even in healthy people who don't experience side effects, we

now know there are all kinds of major disturbances going on. So that's one area I'm very interested in.

Another is how the diversity of this microbiota in the human body is distributed in space, and in time. What's the biogeography of bacterial diversity in the human body? Are there patterns of distribution that tell us something about the physiology of the human body? That tell us something about how communities might be involved in maintaining health and promoting disease at certain sites and not others? Could it be related to why one segment of the intestines becomes inflamed and the one next to it doesn't? Or why one section of the intestinal tract develops a flare-up of Crohn's disease and 10 centimeters away everything is fine? We're trying to look more carefully at whether patterns of microbial diversity predict subsequent disease, or whether subtleties in the patterns of diversity, say, along the length of the bowel might tell us about why this disease occurs in one place not another.

SW: Are there particular diseases in which you think the microflora play a causal role?

Yes, although at this point we're still guessing as much as anything. I do think we'll find differences in the bacterial communities in a number of diseases, although we'll then have to establish whether these differences are responsible for the disease or simply a result of it. That's the causation problem, and it's really not yet adequately addressed for a lot of diseases. If these are going to be important associations, even important causal associations, it will be in settings like inflammatory bowel disease, or chronic gum disease or antibiotic-associated diarrhea. There are number of other conditions where people have speculated that they may be important—irritable bowel syndrome, for instance, which is different from inflammatory bowel disease. There is some evidence for differences in the composition of the microbiota. There's a long list of disease states where some early work or partial data has suggested a link.

SW: What message would you like to give lay readers about your research?

I guess one message is that there is a great deal of microbial diversity in the human body and these are almost entirely all organisms whose presence is not something to fear or be worried about, but rather, something for which to be thankful. To a large degree our microbial inhabitants are contributing to our own health. They are a fundamental part of who we are as healthy, compensated, adaptable living organisms.

The second message might be that the result of all this work—of this whole emerging community of investigators—is that it may have real impact on our ability to recognize early signs of disease and then perhaps our ability to do something about it. Once we understand these bacterial communities better, we'll be able to figure out how to maintain a healthy community picture or restore it if it's already become disturbed. That's also a positive message—that this research may indeed lead to options for positive intervention.

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David Relman's current most-cited paper in *Essential Science Indicators*, with 475 cites:

Eckburg PB, *et al*., "Diversity of the human intestinal microbial flora" *Science* 308(5728): 1635-8, 10 June 2005. Source: *Essential Science Indicators* from Thomson Reuters.

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