Shelters from the Storm
Sex-Based Defenses Against Disease
Why hasn’t infectious disease research reflected fundamental differences in women and men?

Sabra Klein forces a handshake, coughs by way of greeting.

"Would you like one?" she rasps, sharing a bag of mentholated lozenges. "It’s not the flu. I don’t think."

Flushed and weary, Klein plans to retreat early from her office and lab in the Bloomberg School, quarantine herself at home, rest and drink lots of fluids. But not before she infects you, via this article, with the germ of an idea she has long championed: Sex matters. In ways we never fathomed.

It matters, for instance, with the flu that Klein hopes she doesn’t have. Her ongoing studies show that females have a bigger and badder inflammatory response. They don’t just feel worse. They don’t just visit doctors more or complain more. They literally experience worse disease than males. Klein’s talking sex-based biology, not gender issues. (Although "sex" and "gender" often are used interchangeably, sex is biology while gender refers to the social constructs related to one’s sex.) Molecularly speaking, females respond differently to flu than males. They mount a more robust immune response—which sounds like a good thing, until you delve into Klein’s data and see that this heightened immunity contributes to tissue damage and even death. Females respond so differently to immunizations, she concluded, that a woman needs about half the flu vaccine dose of a similarly sized male.

Klein coughs, pops another lozenge and launches into why she loves the flu.

For a dozen years, the assistant professor in the W. Harry Feinestone Department of Molecular Microbiology and Immunology has investigated infectious diseases, first focusing on hantaviruses, then malaria, now flu. The constant throughout her career has been her dogged insistence that sex matters.

That concept, neither new nor original, holds that every cell in us—indeed, every cell in the H1N1-infected mice languishing in Klein’s lab—has a sex. Advocates of sex-based biology contend that maleness or femaleness in humans as well as rodent needs to be considered, compared and contrasted in order to uncover basic biological truths about everything from heart disease and depression to lupus and liver cancer.

Klein’s data always have spoken louder to her than naysayers. As an infectious disease expert working in nonreproductive tissues and cells, Klein sensed for years that her grant submissions or research papers focusing on sex differences didn’t so much pique reviewers’ interest as annoy them. Fellow researchers who ignore sex differences have distinguished careers and mountains of data invested in their way of doing things. Some told her flat out: Sex did not matter.

Still, she stayed the course, giving sex differences center stage instead of sloughing them off. She used both male and female animal models in her hormone-centric studies. She manipulated estrogens and testosterone, surgically removing the bird-seed-size ovaries and testes of mice, and then put hormones back, always looking for cause-and-effect relationships between sex and disease. She analyzed her results by separating the sexes in the statistics instead of lumping males and females together in one big androgynous data set, as was—and still remains—conventional practice. (In top-tier journals, it’s common for authors of clinical studies to demonstrate demographic correctness in Table 1, showing 50 percent of their subjects were male and 50 percent female. However, after that obligatory nod, the breakdown by sex simply goes away, never to appear again in Tables 2, 3 or 4, Klein says: “There’s no more mention of sex. No statistical comparison. It’s sooooo frustrating!”)

Klein saw firsthand, time and again, that sex was remarkably relevant to her research on animals’ susceptibility to infection and their response to pathogens. Indeed, sex often was the only difference between those that recovered and those that succumbed to disease. With influenza, for example, when she gave male and female mice a standard dose of virus, none of the females would survive past two weeks, while more than half of the males would recover, surviving the infection. For many infectious disease mouse models, if fewer than half of the mice of a particular strain survive an infection, then that strain is defined as “susceptible” and if more than half of the mice live, that defines the strain as “resistant.” But while working with two sexes of the same strain, Klein noted stark differences.

There are other people—not so many in infectious disease but more studying the
"Sabra was the prepared mind in the right place at the right time."

heart and brain—who are as convinced as Klein that males and females differ in their basic physiology and, therefore, in the susceptibility to and progression of diseases. Arthur P. Arnold, a professor and chair of physiological science at UCLA who assumed editorship of the just-launched journal Biology of Sex Differences, has been doing sex-based biology since the mid-1970s when he was a neurophysiology postdoc at The Rockefeller University. He realized early on that gonadal hormones explained only part of the reason a brain structure in male songbirds was six times larger than in non-singing females. Some sex differences, he has since discovered, are the result of direct action of genes encoded on the sex chromosomes: XX and XY cells differ functionally because of the action of X and Y genes intrinsic to the cells.

The fact that there are sex differences in disease implies that one sex has something protective about it, Arnold says. If that something could be enhanced or modified, it might affect the disease. That something might make an attractive drug target, for instance, and only a lack of understanding of the biological basis of sex differences in disease keeps us from hitting that target with new therapies.

Researchers like Arnold and Klein quietly celebrated a milestone 10 years ago when the Institute of Medicine issued a report ("Exploring the Biological Contributions to Human Health: Does Sex Matter?") concluding that every cell has a sex, and therefore sex matters in health "from womb to tomb."

Heartening as that was, it more or less preached to the choir, Klein says. That choir subsequently formed a new academic society—the Organization for the Study of Sex Differences—to promote the interests of this emerging field, not least of which was to address the fact that only a pitance of NIH grants supported the study of sex differences.

Klein, with a small cadre of colleagues across various disciplines, proposed in 2008 to establish a center for the study of sex-based biology at Hopkins. Helping lead that effort was Pam Ouyang, a cardiologist based at Bayview Medical Center, who says, "Men and women are different in lots of ways that we don't necessarily understand yet because we don't study them. I thought it would be really nice to have a place where general conversations about sex differences could be discussed with people from various spheres of knowledge who would approach questions—such as why are men's and women's risks of heart disease different—from all angles."

Ouyang, no doubt would have been intrigued by DeLisa Fairweather's perspective on autoimmune heart disease in men. A staunch advocate of sex-based biology who is based in the Bloomberg School, Fairweather is teasing out how inflammation induces chronic conditions in males and females.

Despite their common interests, Ouyang and Fairweather were not yet destined to meet. The proposal for a center was denied. The upstart field of sex-based biology limped along on its uphill trek.
H1N1 threw her—and sex-based biology—into the limelight.”  —Florence Haseltine

Then the flu hit.

Not just any old flu, but the H1N1 pandemic of 2009. The fact that it hit in the midst of a panic about vaccine shortages proved fortuitous for Klein. Steeped in sex-based biology à la influenza, she was ready with a kill shot. She leveraged interest in the pathogen, finessing a low-grade professional buzz about sex differences into a very public debate. Flu, once and for all, confirmed the legitimacy of her convictions.

“Sabra was the prepared mind in the right place at the right time,” says Florence Haseltine, MD, PhD, director of the Center for Population Research at the National Institutes of Health and a founding member of the Organization for the Study of Sex Differences. “H1N1 threw her—and sex-based biology—into the limelight.”

Klein, a mother of two girls, promptly coauthored an op-ed (“Do Women Need Such Big Flu Shots?”) published in The New York Times in October 2009. She wrote, “In all likelihood, we’d have a better H1N1 vaccine—and more of it—if in our preparations we had accounted for the biological differences between men and women.” That article sparked the WHO to enlist Klein’s help in preparing “Sex, Gender and Influenza,” a report issued in July 2010 that examines the 2009 H1N1 pandemic through the prism of sex differences in immunology.

Meanwhile, Klein also coauthored a review that appeared in the May 2010 edition of The Lancet Infectious Diseases (“The Xs and Ys of Immune Responses to Viral Vaccines”) that re-examined published data from a high-profile paper by analyzing it according to sex. The review revealed conclusions strikingly different from those of the original authors who had ignored sex. When Klein re-analyzed the genomic data by sex, she found that the transcriptional activity along immunological pathways—pathways that supposedly predict long-term protection following, in this case, yellow fever virus vaccination—was 10-fold higher in samples collected from female than from male volunteers, suggesting that females may be better protected than males.

About that same time, a landmark reference book Klein co-edited was published: Sex Hormones and Immunity to Infection.

Amid her publishing flurry, Klein accepted invitations to speak locally and abroad. She described how her female mice were mounting inflammatory responses up to a hundred-fold higher than males in the first week after flu infection. She cautioned audiences against assuming that a bigger immune response is better. Take the 1918 flu or the avian flu, for example: They caused profound sickness and death, she explained, not because of out-of-control viral replication but because the human hosts—and hostesses—initiated excessive immune responses to those pathogens.

Despite preaching about a pervasive lack of consideration for sex differences in the design of scientific studies, and therefore in the analyses of data, Klein never expected people to suddenly fixate on sex differences. She just wanted them to pause and question the assumption that males and females weren’t going to be different.

And then, out of the blue, a Flopkins
“In biomedical science, the dogma is that there are no differences between men and women. People like myself who design studies looking at both sexes are left with that uphill battle of challenging the dogma.”

—Sabra Klein

physician investigating Lyme disease contacted Klein about analyzing his patient data according to sex. On the heels of that request, a scientist from New York University conducting malaria research in Peru sought her out, wanting to discuss intriguing sex-based trends. Most recently, a Harvard researcher offered to share unpublished epidemiological data and asked to pick her brain about sex differences in flu immune response.

“Flu is of such great public health importance,” Klein says, “that it puts all of this sex-based biology business in a context people suddenly care about.”

Sex differences were not a part of John Aucoott’s original research plan when he set out three years ago to study the natural history of Lyme disease. In his field, there was no precedent for separating out male cases and female cases. It simply hadn’t occurred to him, a Hopkins-trained physician and fellow in infectious disease, that sex had anything to do with his patients’ biological reactions to a tiny tick bite.

Aucoott is the go-to guy in the Mid-Atlantic region for Lyme patients with chronic post-treatment issues. Considering that Lyme is an emerging epidemic, and he’s based at its epicenter, Aucoott is busy. A steady stream of acutely ill patients sporting telltale rashes show up daily at the urgent care center next door to his office in suburban Baltimore. From their ranks, he recruits study subjects “by the gobhs.” Already he’s beginning to glean valuable information about the “normal” human immune response to an acute infectious disease over time.

“Actually, we’re going to get a two-fer,” Aucoott enthuses. “We are going to describe the normal human response in men and in women. It can’t be the same. Why would it be? I’m convinced of that now. I’m a believer.”

Aucoott credits his conversion to study coordinator Alison Rebman. It was her key observations that led to finding differences in antibody responses between men and women—and, ultimately, to Sabra Klein. About a year ago, she noticed significant trends that appeared to vary by sex and wondered if anyone had ever looked at sex differences and Lyme before, so she dug into the scientific literature. “I came across a review Sabra Klein had written about viral infection, and then a chapter of her book. I kept coming across her name, and after reading a few more articles, realized she’s at Hopkins!” Rebman says.

She contacted Klein, whose excitement about their Lyme findings was, well, infectious. “Sabra talked about how the female immune system is designed differently than a male’s,” Aucoott says. “It has to do with all these crazy immunologic maneuvers to tolerate being pregnant. She’s helping us to think about how to design our study and how to analyze the data.”

Rebman’s initial discovery—that sex may affect the antibody response measured by the diagnostic test—landed her and Aucoott in unexplored territory. It was known that the standard test for Lyme is not very sensitive, and apparently it can be even less so for women. Possibly, many, many more women go undiagnosed than ever suspected.

People who don’t get treated are known to develop months or years later a condition characterized by big swollen knees; it’s known as late Lyme arthritis, and twice as
many men get it as women, according to Aucott.

“We know that equal numbers of men and women get Lyme to begin with, and twice as many men get late Lyme arthritis, so what happened to all the women?” Aucott asks. “Did they all just get better without treatment?”

He thinks not. Rather, he speculates that maybe the women who didn’t make enough antibodies to fit the diagnostic criteria for Lyme in the first place ended up getting diagnosed with something else: fibromyalgia, for instance.

Anecdotal information, now backed up by sex-based biology, informs his hunch: Acute Lyme disease is easily treated with an antibiotic. The rash and other symptoms disappear. However, 20 percent of people post-treatment—the majority of them women—develop a fibromyalgia-like syndrome some months later. The overall ratio of women to men with fibromyalgia is 7:1.

Aucott sees lots of patients who report having felt fine before their bout with acute Lyme, then recover, only to develop a vague constellation of disabling symptoms sometime later. The majority of them are females.

“These are people who are told by their physicians that they are ‘just depressed.’” Rehman says. “Well if they weren’t depressed before the rash, there’s got to be more to it. That can’t be the end of the story.”

Sex differences complicate the story. No doubt about it. Marc Lipsitch, director of the Center for Communicable Disease Dynamics at the Harvard School of Public Health, will attest to that.

His lab was looking for evidence that early exposure to certain strains of influenza proved protective against infection in the 2009 pandemic. Lipsitch’s group noticed, as others had, that older people seemed at lower risk of being a confirmed flu case in 2009. Why would that be? Did they have antibodies from immune responses earlier in life? Did they have fewer children at home and therefore were less exposed to school kids, known transmitters of flu?

“We thought it would be important to know differences between men and women in the context of this drop-off in risk,” Lipsitch says, “because you might expect a bigger drop-off in women than men if it was due to exposure.” When his team separated out the data by sex, they realized that although the drop-off in risk is much stronger in women, it does not appear to be due to any lack of contact with flu-infected people.

Sabra Klein came to mind: Lipsitch recalled seeing a poster at a conference by one of Klein’s students, showing how the immune system behaved differently according to sex.

“We thought she’d be interested in our unpublished data,” he says, admitting that he originally intended to ignore sex. “We actually had hoped to reject the idea that what we were seeing had something to do with sex differences in the immune experience.”

“But it was really helpful that Sabra pushed us to look at both age and sex at the same time and make sense out of data of this sort, even though now it’s a more complicated story.”

OraLee Branch, an assistant professor of medical parasitology at New York University, studies the immune response to malaria infection. When her students, who
accompanied her to Peru for a field investigation, noticed sex-based trends in their data, she scrambled to find anything even remotely related in the malaria literature, finally unearthing a paper Klein published in 2006.

"I right away picked up the phone and explained to her what we were finding," Branch recalls. "I wondered if she had followed up, if there was more information. Her work had since veered toward viral pathogens. But still, she offered to help."

One of the things Branch had spent time investigating was differences in malarial symptoms based on differences in exposure.

Now she wants—no, she needs, she says—to go back into her data to reanalyze it, taking sex differences into account. "It's really opened up a can of worms, it has," Branch says. "We might find immunity is developing better in males than females, or maybe the other way around. But pretending sex differences don't exist is just going to obscure the real mechanisms we're all trying to find. Pretending differences don't exist is not the answer."

All was unusually quiet throughout the Bloomberg School of Public Health on the snowy holiday commemorating Dr. Martin Luther King.

Michael Coronado—winner of the Florence P. Haseltine Award for young investigators at the 2010 annual meeting of the Organization for the Study of Sex Differences—was hard at work in DeLisa Fairweather's lab, however.

First, he was extracting blood from male and female mice that were infected with the same virus but producing different levels of cytokines—the signaling molecules that cells generate to communicate with each other. Then, he was removing their hearts to study the diseased tissue. Later, on other live mice, there were gonadectomies to perform.

By de-sexing the males and females, he was attempting to make them biological equals, at least in terms of the type of heart disease he's studying. It's apparent that testosterone drives the disease. But it also could be that estrogen protects from it. Or both could be true.

One thing's for sure: Male and female mice are anything but equals in terms of their hearts, as the males' floppy, scarred organs clearly show.

Some investigators would justify using only male mice to study disease that affects mainly males. They'd argue that it's expensive and inconvenient to deal with female rodents because it means having to account for hormonal cycles.

For that matter, lots of researchers who study equal opportunity diseases such as cancer don't use both sexes of mice either, no matter that half the population whom the research is ultimately meant to serve happens to cycle, too.

The converse is also true. Lots of researchers studying so-called women's diseases such as lupus and multiple sclerosis use only female mice and therefore risk missing half the picture.
Both males and females always are used for comparative purposes in the Fairweather lab where sex differences have been shown to drive autoimmune disease.

Autoimmune diseases are notoriously lopsided in terms of whom they strike. Thyroid disease affects mainly women while dilated cardiomyopathy (the chronic type of heart disease that Fairweather’s studying) affects men more.

It’s not simply good practice to acknowledge sex differences, it’s outright dangerous to ignore them, says UCLA’s Art Arnold: “Treating one sex like the other may be as inappropriate as treating a child like an adult. The equitable treatment of females and males requires an understanding of their differences.”

When Sabra Klein gave presentations early on in her career and talked about the differences she saw between the sexes, she was invariably followed by a more senior colleague who would not mention any differences between males and females. Inevitably, an audience member would ask if he saw the same types of differences that Dr. Klein reported.

The answer, Klein recalls, always was curt and final: No. Absolutely not.

“That would be it, with no further explanation,” Klein says. “And I’d feel everything sink. I wasn’t born with thick skin. I’d take it personally and worry: What are people trying to say about me, my data, the quality of my work?

“But as soon as I’d get back into the lab, into what we were doing, I’d get over the intimidation because I was excited by the research. I believed in it.”

It didn’t hurt that senior faculty in her department believed in her work as much as she did: “Diane Griffin, Al Scott and Greg Glass had a lot to do with my ability to stand up and be that lone voice in the wilderness. They took a risk hiring me. They told me I needed to lay claim to a field.”

These days, the very same people who made presentations after Klein and disavowed sex differences now are sending their data to her, reporting that they too are seeing trends. They now allow Klein to present their unpublished data in talks, which is a giant leap forward. But out there in the published world, sex differences remain largely ignored. “When you put your data out there, you are choosing to enter a debate,” Klein says. “It’s not some definitive conclusion; the book is not closed, the story not over. You put yourself out there to be judged, and to be open to people’s interpretations. I am always working to hone my arguments, to improve on my logic.”

But as the long, cold flu season gives way to spring, Sabra Klein senses a thawing of attitudes toward sex-based biology. Her team recently resubmitted a research paper for consideration by a prestigious journal. Because the reviewers’ comments and concerns had been so constructive, Klein has reason to believe that she’ll hear good news back soon: “I think they might actually be rooting for us.”

EXTRA

Sabra Klein describes “his and her” heart attacks: magazine.jhsph.edu/extras