Them and us: microbes and their human host.

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Thank you Haemish for your generous introduction. I suspect I should sit down while I am ahead!

Them and us: microbes and their human host: in three sections.

- The microbes that live on us, all of us; our normal flora.
- Those that invade many of us, though not all of us and then remain persisting, alive within us.
- And finally, those that have invaded our ancestors many years ago and have now become part of us, all of us, part of our very selves.

I gave many lectures to medical students and others about our normal flora from 1970 to the early 2000s. What I said didn't change very much over the decades: there was little really new. Our normal flora is the millions of organisms that live on us: skin, mouth, gut (gastro-intestinal tract) and vagina. Those in the gut have the same relationship to us as those on our skin. It is, after all, possible to travel from mouth to anus without crossing a tissue surface: simply inside a tube. You may not especially want to do that, but that's another matter. We say these organisms colonise us, rather than infect us, to use the correct terminology. And most now know that all creatures, to use the David Attenborough generic term, have a colonising flora: dolphins, armadillos, kauri, kiwi and cotoneasters. The cells of our gut normal flora outnumber our own human cells at least tenfold. They are estimated to weigh over a kilogram, about the weight of a human brain. And despite your personal convictions, gentlemen, appreciably more than both testes. So, to that sexist assertion that "*He's more balls than brains*", the appropriate response is "*That's not correct, he's actually more germs than balls*".

It has been known in a quite simple way for many years that the organisms of our normal flora, predominantly bacteria but including fungi, protozoa, mites and some viruses are an effective barrier against invading pathogens, disease causing microbes. It is simply very hard for potential invaders to infiltrate themselves into the physically and metabolically closely knit, evolutionarily integrated normal flora. This

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characteristic makes it equally very hard to implant '*good bacteria*' into these tight, balanced ecological niches. Which takes me to the Probiotic Industry, now infesting this country. '*Probiotic*' is a very strange and to my mind inappropriate name. '*For life*'? Nevertheless, probiotics are now precisely defined scientifically as '*live organisms which when administered in adequate amounts, confer a health benefit on the host*'. For the vast majority of claims made in this area, there are no decent data at all to support their use, and particularly none in relation to taking them routinely with antibiotics '*to restore normal flora'*. Having said all that, probiotics as defined are a very appealing idea and there are now a small number of special situations when a particular defined probiotic has indeed been shown to confer a health benefit. For the most part however, the advertising hype has way outstripped the proven science.

The Human Genome Project drove the development of increasingly clever ways of sequencing genes. Then by linking the genetic data with the powerful capacities of modern computers to handle enormous data sets, it became possible to identify all the individual microbial genes in complex microbial communities such as, for example, in the human large bowel. Scientists then go backwards, in a sense, and can confidently predict the organisms that carry those genes. '*The Human Microbiome*' is the new term now used for '*our normal microbial flora, its genes and their interactions*'. It is not really different from our normal flora, but more detailed and with a broader and different emphasis. The Human Microbiome Project was formally started in 2005 and reported in 2012. In the last months, The Guardian Weekly, Time, Scientific American, The National Geographic and The New Yorker, to my knowledge, have all run lengthy articles on this topic of The Human Microbiome and its potential significance.

In the first instance The Project has confirmed what has long been known: that there are very many more organisms in our microbiome than have been seen or cultured up till now. For those who like numbers, there are in the large bowel over 2000 different bacterial species and they carry 3.5 million genes. This technology and the accumulating data have spurred a resurgence of interest and research in this area. As examples, increasing numbers of very precisely determined molecular mechanisms are now being demonstrated for interactions between particular members of our microbiome and our immune system, in terms of its normal development, its maturation and its life time behaviour, as well as potentially in immune mediated diseases.

Bacteria influence stomach pH normally and they effect the release of a stomach hormone, ghrelin, which regulates appetite. They may have roles in inflammatory bowel disease and in the common condition of irritable bowel syndrome. There are differences in the gut microbiome of obese people and of diabetic people, compared with normal people. A lot of this data is very early and I don't wish to overstate its human relevance. It is however an area of increasing research interest and time will determine just how important some of these findings are. Watch this space: my advice to medical colleagues is not to reflexly write it all off as far fetched.

Now, to the second part of my talk. This is about a small number of organisms which infect us, that is, breach a body surface and enter our tissues. The outcome of any and all infection ranges from no symptoms at all, the most successful outcome from our and our immune system's point of view, through symptoms minor or major and sometimes to death. These outcomes depend on the particular microbe and the human host at the time. Even in pre treatment times, most infectious diseases resulted in organism death and human survival. But a small minority of infecting invaders were rarely killed, ending up sequestered in some organism specific anatomical site where they went to ground, persisting in us, latently infecting us as we say, seemingly relatively untroubled by our immune system.

The bacteria that cause tuberculosis, *Mycobacterium tuberculosis* do this: maybe 15% of us, myself definitely included, likely have *M. tuberculosis* living in some lymph nodes somewhere, most likely in our chests, this very night. *Toxoplasma gondii,* the protozoan which causes toxoplasmosis and which has cats or other felines as its primary host, does this too. Again, probably about the same percentage of us in this room have this organism persisting alive in our brains, our skeletal muscles in general and in our heart muscle.

And my last example is the Herpes virus family. All eight members of this family that infect us, live latently in us in one way or another. As examples, *Herpes simplex*, either the one that causes cold sores, or the genital variant and *Herpes varicella zoster*, the one that causes chicken pox and later in life, shingles (zoster), persist in nerve cells in our spinal cords. Once infected, *Herpes simplex* in particular, at varying frequencies, tracks down the segmental nerves arising from the nerve cells they persist in to cause the characteristic skin sores at the ends of those particular nerves. And we call these episodes, recurrences and they obviously provide, for the virus, a potential for spread to a new human host. Probably 80% of us here have *Herpes simplex* in the posterior root ganglion of our Vth cranial nerve in our heads and maybe 25% of us, again, myself included, get recurrent cold sores. Nearly all of us, certainly more than 90%, have *Herpes varicella zoster* in nerve roots somewhere in our spinal cords and carry the unpredictable risk of shingles as we age.

So, another '*them and us*' association: persistence, latency and sometimes relapses.

Now the third and final part: those evolutionary microbial descendents found within all the cells of everyone in this room, normally and usually. This third section is in two parts.

Firstly; all our cells have a range of structures within them: a nucleus (though to be precise, not in circulating red blood cells), cytoplasm, filaments, canals, the Golgi apparatus and so on which are involved in the chemistry, the structural integrity, the overall metabolism of the cell. One such structure is the mitochondrion: it looks

rather like a tiny capsule. There may be only a few, or there may be thousands in a particular cell. They provide the source of chemical energy which drives all intracellular processes. Without them, we could not and would not, be.

From the 1970s it was suspected by a small group of biologists led by a combative American called Lynne Margulis (who incidentally was married for quite a time to the astronomer and astrophysicist Carl Sagan) that mitochondria were pared down, diminished bacteria. While the arguments for this view were very sophisticated, they boiled down to the old adage that "*If it looks like a duck, walks like a duck and quacks like a duck, it is a duck*." She was dumped on and her articles rejected frequently by the high priests of biology of the time for decades, but lived long enough, until 2011 in fact, to be entirely vindicated. With gene sequencing it has been shown that mitochondria, intracellular components of all animals more sophisticated than amoebae, and of plants, are derivatives of bacteria called *Rickettsia*.

Free living *Rickettsia* still cause much infectious disease in the world today: the most famous, though less common today, is epidemic typhus caused by *Rickettsia prowazeckii*, spread between humans by lice when hygiene breaks down catastrophically. It is reckoned by some that more French soldiers died of epidemic typhus during Napoleon's ill fated march to Moscow and back in 1812, than died from fire arms or the cold. And Ann Frank along with thousands of others, died of this disease in the second World War in the early 1940s.

It was Lynne Margulis' opinion, now widely accepted by evolutionary biologists, that some ancient amoeba like organism, more than one billion, one **billion**, years ago, ingested a rickettsial ancestor, which survived inside the amoeba, becoming an internalised supplier of chemical energy, allowing the development of much larger cells and eventually, multicellularity. As an aside, the '*ingestion*' and incorporation of various small microbes by larger organisms, and their continued survival inside those larger cells is well recognised in biology. It is in no way a bizarre nor unusual event. So, a critical intracellular bacterial contribution to life and eventually to human life.

Now to the second part of this section: as the Human Genome Project was unrolled, there were many surprises. It was widely expected that we, *Homo sapiens*, as we so humbly call ourselves, as has been said so many times, would have the most total DNA in our chromosomes in our nuclei, or if we didn't, surely we would have the greatest number of genes. After all, look at our complexity. We were wrong on both counts. For example water cress and cocoa plants, not well known for their high IQs, though we may do them an injustice, have 30,000 to 40,000 more genes than we do; we have '*only*' about 20,000 and the gold medal for the largest genome to date remains in the possession of an amoeba, *Amoeba dubya*. If that all worries you, remember we do have the riposte, "*Well, maybe, but Shakespeare, Beethoven and Einstein were Homo sapiens!*" Size is indeed, not everything.

And as molecular and evolutionary biologists and virologists have continued to pick away at the human genome and those of more and more creatures, large and small, all sorts of things have turned up. Only about 5-6% of '*our*' nuclear genome i.e. all the DNA in our nuclei, is actually genes and about another 5-6% codes for switches which switch the genes on and off. Another 8% of our genome is undeniably viral DNA, remnants of retroviruses, relatives of HIV, the *Human Immunodeficiency Virus*, the cause of AIDS. HIV is a retrovirus. Retroviruses are uniquely able to stitch themselves directly into our genomic DNA and their remnants are now found widely in the nuclear DNA of the animal kingdom. Depending on just what experts decide to classify as potential retroviral sequences in our genome, some of them argue that maybe as much 50% of our genome is retroviral in origin. A very curious thought. Our '*uniqueness*' is very definition dependent.

So, while just a few years ago we thought that HIV was the only retrovirus to infect us, we now know that retroviruses have been infecting our very ancient evolutionary ancestors, time and time again, over millions of years. You will be pleased to hear that these persisting genomic retroviral sequences are altered, mutated, degenerate, non-functioning pieces of DNA, at least in an infective, disease capable, fully competent, pathogenic retrovirus sense. It is however, on the other hand, equally clear that quite a few of these retroviral sequences help regulate the expression of some useful human genes. Evolution, as has been said, is an extraordinary '*tinkerer*' process and will use and exploit any material and mechanisms available to it. Our genomic DNA has co-opted, for want of a better word, some of this retroviral DNA and uses it. Perhaps the most famous example is that of two proteins called syncytin 1 and 2 which play critical roles in the formation of the human, and many of our relatives' placentas. A syncytium is the technical term given to an aggregation of cells fused together by the breakdown of cell walls, resulting in a large mass of cytoplasm now containing many nuclei. Several viruses can do this, including some herpes viruses as well as retroviruses and you can probably see intuitively how being able to do that might help you, if you are a virus, to spread from cell to cell. There is an anatomically long recognised, single cell thick, syncytial layer in the human placenta which separates maternal blood and antigens from foetal blood and antigens, fundamental to preventing the potential immune mediated rejection of the foetus by the mother. That layer has long been called the syncytiotrophoblast. We now know that the development of this layer is directly under the influence of the syncytins.

So, a co-opted retroviral gene now coding for two human proteins at the very heart of the development of the placenta in humans and many other (though in another twist I have not time for) but not all, placental animals.

A brief diversion from retroviruses and DNA to give you all a rest! I personally love coincidences, long being a believer in '*cock-up'* rather than '*conspiracy*' theories of much, maybe most, human activity. The last scientific paper that Charles Darwin published before his death in 1882 was about a barnacle. Remember he was the barnacle expert *par excellence* and spent about 10 years dissecting and classifying them in a *tour de force* which I understand, to this day, still underpins their

classification. The man who sent him this particular barnacle, which he had found attached to the leg of a fresh water beetle, was an amateur naturalist and shoemaker, Walter Dowbridge Crick: and yes, of course, he was the grandfather of Francis Crick who, along with other scientists, some well recognised and honoured and some less so, but most famously the American James Watson, determined and published the structure of DNA in 1953. Nowhere near six degrees of separation. And literally just a few weeks ago, after Francis' death, the Crick family sold his 1962 Nobel prize to one Jack Wang, CEO of Bromabie, a regenerative medicine tech company in Silicon valley, for 2 million dollars. There you go!

Returning now for the last time to our own retroviral remnants which litter our genome and the genomes of so many of our close and distant evolutionary ancestors; clues to what this might be all about come from koalas. If you think Australian cricketers and rugby players are having a tough time of it recently, spare a thought for koalas. For over a hundred years they have been sickening and dying in an epidemic of their own version of AIDS caused by their own koala retrovirus. This epidemic began in the NE corner of Australia, nearest to Asia, undoubtedly the original source of this epidemic and has been slowly working its way down the eastern seaboard of Australia and now across towards South Australia. Indeed the only uninfected koalas are now found only in South Australia. However some have been found with this virus stitched into the genome of every cell in their bodies, but the virus now seemingly disabled and incapable of disease. Presumably this has occurred by the chance '*infection*' or uptake of the retrovirus into a koala ovum or sperm cell (uptake into sperm cells has been found in male koalas), which has subsequently become the fertilised or fertilising cell for a new koala foetus, which will therefore have the retrovirus in every cell of its body. While all the dots for the mechanisms of these events are not yet fully connected, the notion is that we are watching the evolution of a pathogenic, intermittently infecting retrovirus into a benign component of the koala genome in every cell of its body. The further supposition is that this process, or some close replica of it, is the story of all the multiple incursions of retroviruses into our and our ancestors' DNA, both recent and distant.

So: there is really, almost, no '*them and us'*; we are inextricably linked in extraordinary ways. Louis Pasteur, one of the founders of the science of microbiology, said towards the end of his life, in 1895, "*Mesdames and messieurs: les microbes auront le dernier mot*". "*Ladies and gentlemen, microbes will have the last word*." But even in his wildest dreams, he could never have imagined it would be like this.

Thank you.