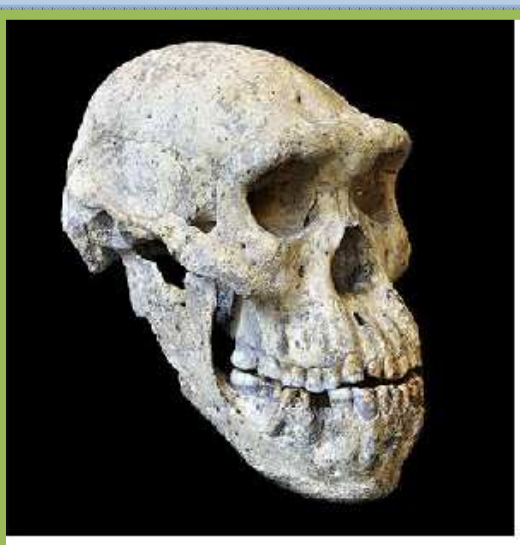
"A BOOK, TOO, CAN BE A STAR, A LIVING FIRE TO LIGHTEN THE DARKNESS, LEADING OUT INTO THE EXPANDING UNIVERSE."

— Madeleine L'Engle



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About the cover: *The image on the cover was taken from Google Images. The cover illustration represents that man prove themselves to survive in the nature. Year up to year people fight against disease and succeed in most cases.*

PREFACE

Magazines constitute an important source of information in literate societies. Their importance lies in the current information they carry. ***WTM, Bangladesh*** published an e-magazine in the month of **June-July, 2014**.

I am very grateful to ***Mr. Saumyadip Sarkar*** *(Managing Director, WTM)* and ***Mr. Swapnil Vichare*** *(Chief Editor, WTM)* for encouraging me in this work. My special thanks to our magazine editor ***Md. Mehedi Hasan Magnet***, for his pleasant co-operation and valuable discussion. One special thanks to ***Toufeequl Islam Nirob*** for excellent assistance in this work.

Thanking You,

Md. Golam Moktadir Khan

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The level of awareness and taste of the reading public is changing, the demand and thirst for information is on the high. So, **WTM, Bangladesh** published their 3rd E-magazine (**Volume - 2, Issue - 1**), **June-July, 2014**.

I am very grateful to all colleagues for encouraging me during this work.

Thanks to Everybody,

Md. Mehedi Hasan Magnet

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Content

Abstract

MICROBIOLOGY- PAVING A LONG WAY

When you hear the words “germ,” “bacteria,” and “virus” you might cringe, running for the nearest sink to wash your hands. These words may bring back memories of when you caught a cold or the flu—never a pleasant experience. “Wash the germs from your hands!” That was the cry of every mom who knew that hand washing is the best way to prevent sickness. Most kids balked at hand washing simply because they couldn’t see the germs on their hands. Germs, bacteria, viruses and other microscopic organisms are called microorganisms, or microbes for short. Microbiology has been an important science for the past 100 years in that it has provided the means to control a number of infectious diseases and the experimental systems for the development of molecular biology. Microorganisms can be found in every ecosystem and in close association with every type of multicellular organism. They populate the healthy human body by the billions as benign passengers and even as participants in bodily functions, for example, bacteria play a role in the degradation of intestinal contents. Prokaryotic cells are bacteria cells and eukaryotic cells are cells of animals, plants, algae, fungi, and protozoa. Each carries out the six life processes that all living things have in common. There are thousands of microbes and no two are identical, but many have similar characteristics. Microbiologists have spent years carefully observing microbes and organizing them into groups by their similarities. Bacteria are one of the most common microbes that you encounter. Some bacteria cause disease and other bacteria help you live by aiding in digestion. There are many different kinds of bacteria; however, all bacteria can be grouped into four divisions based on the characteristics of their cell walls.

The microscopic world was first visited in the late 1600s by the Dutch merchant and amateur scientist Antoni van Leeuwenhoek. He was able to see living microorganisms by using a single-lens microscope. We’ve come a long way since Van Leeuwenhoek’s first visit. Today

scientists are able to see through some microbes and study the organelles that bring them to life. It wasn't until the Golden Age of Microbiology between 1857 and 1914 when scientists such as Louis Pasteur and Robert Koch made a series of discoveries that rocked the scientific community. During this period scientists identified microbes that caused diseases, learned how to cure those diseases, and then prevented them from occurring through the use of immunization. Scientists were able to achieve these remarkable discoveries by using culturing techniques to grow colonies of microbes in the laboratory. Once microbes could be grown at will, scientists focused their experiments on ways to slow that growth and stop microbes in their tracks- killing the microbe and curing the disease caused by the microbe. Culturing microbes is central to the study of microbiology. Microbes in our intestines are beneficial to us as long as they remain in our intestines. Microbes need nutrients to grow- chemical nutrients such as carbon, hydrogen, nitrogen, and oxygen. However, not all microbes need the same chemical nutrients. For example, some require oxygen while others can thrive in an oxygen-free environment. Just like us, microbes inherit genetic traits from their species' previous generations. Genetic traits are instructions on how to everything to stay alive. Some instructions are passed along to the next generation while other instructions are not. Who we are and what we are going to be is programmed into our genes. The same is true for microbes. This genetic information is encoded into DNA by the linking of nucleic acids in a specific sequence. Genetic information can be reordered in a process called genetic engineering. Inside our body there is a war going on. An army of B cells, T cells, natural killer cells, and other parts of our immune system are on the defense. These cells seek microbes to rip apart before any of them can give us a runny nose, cough, or that dreaded feverish feeling. The immune system is our body's defense mechanism: Its "soldiers" surround, neutralize, and destroy foreign invaders before they can do harm.

The uniqueness of microorganisms and their often unpredictable nature and biosynthetic capabilities, given a specific set of environmental and cultural conditions, has made them likely candidates for solving particularly difficult problems in the life sciences and other fields as well. The various ways in which microorganisms have been used over the past 50 years to advance medical technology, human and animal health, food processing, food safety and quality, genetic engineering, environmental protection, agricultural biotechnology, and more effective

treatment of agricultural and municipal wastes provide a most impressive record of achievement. Many of these technological advances would not have been possible using straightforward chemical and physical engineering methods, or if they were, they would not have been practically or economically feasible. Nevertheless, while microbial technologies have been applied to various agricultural and environmental problems with considerable success in recent years, they have not been widely accepted by the scientific community because it is often difficult to consistently reproduce their beneficial effects. Microorganisms are effective only when they are presented with suitable and optimum conditions for metabolizing their substrates including available water, oxygen (depending on whether the microorganisms are obligate aerobes or facultative anaerobes), pH and temperature of their environment. Meanwhile, the various types of microbial cultures and inoculants available in the market today have increased rapidly because of these new technologies. Significant achievements are being made in systems where technical guidance is coordinated with the marketing of microbial products. Since microorganisms are useful in eliminating problems associated with the use of chemical fertilizers and pesticides, they are now widely applied in nature farming and organic agriculture. Environmental pollution, caused by excessive soil erosion and the associated transport of sediment, chemical fertilizers and pesticides to surface waters and groundwater, and improper treatment of human and animal wastes has caused serious environmental and social problems throughout the world. Often engineers have attempted to solve these problems using established chemical and physical methods. However, they have usually found that such problems cannot be solved without using microbial methods and technologies in coordination with agricultural production. New developments in biotechnology and environmental microbiology indicate.

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THE ORIGIN OF CELLULAR LIFE

When life arose on Earth about 4 billion years ago, the first types of cells to evolve were prokaryotic cells. For approximately 2 billion years, prokaryotic-type cells were the only form of life on Earth. The oldest known sedimentary rocks found in Greenland are about 3.8 billion years old. The oldest known fossils are prokaryotic cells, 3.5 billion years in age, found in Western Australia and South Africa. The nature of these fossils, and the chemical composition of the rocks in which they are found, indicates that these first cells made use of simple chemical reactions to produce energy for their metabolism and growth. The primitive earth's atmospheric gases, such as ammonia (NH_3), hydrogen (H_2) and hydrogen sulfide (H_2S) could be oxidized to produce energy that allowed conversion of CO_2 to cellular (organic) material. As organic material developed, it became the substrate to support the growth and metabolism of other cells that use simple organic compounds as their source of energy. The use of inorganic chemicals as a source of energy is called chemolithotrophy; the use of organic chemicals as energy sources is called chemoheterotrophy. Thus, chemolithotrophy and chemoheterotrophy were the first two types of metabolism to evolve. An important group of Archaea that were involved in this process were the methanogens, which grow by using H_2 as an energy source and CO_2 as a carbon source, resulting in the production of the simplest of all organic molecules, methane (CH_4). Archaea and bacteria probably arose from a universal ancestor but are thought have split early during the evolution of cellular life into the two groups of procaryotes that we recognize today. Photosynthesis (metabolism which uses of light as an energy source) developed in bacteria about 3.2 billion years ago. The first type of photosynthesis to appear is called an oxygenic photosynthesis because it does not produce O_2. An oxygenic photosynthesis preceded oxygenic photosynthesis (plant-type photosynthesis, which produces atmospheric O_2) by half a billion years. However, oxygenic photosynthesis also arose in procaryotes, specifically in a group of bacteria called cyanobacteria, and existed for millions of years before the evolution of plants. As molecular oxygen (O_2) began to appear in the atmosphere, organisms that could use O_2 for

respiration began their evolution, and "aerobic" respiration became a prevalent form of metabolism among bacteria and some Archaea.

Microbes and the origin of life on earth

Scientific evidence suggests that life began on Earth some 3.5 billion years ago. Since then, life has evolved into a wide variety of forms, which biologists have classified into a hierarchy of taxa. Some of the oldest cells on Earth are single-cell organisms called archaea and bacteria. Fossil records indicate that mounds of bacteria once covered young Earth. Some began making their own food using carbon dioxide in the atmosphere and energy they harvested from the sun. This process (called photosynthesis) produced enough oxygen to change Earth's atmosphere. Soon afterward, new oxygen-breathing life forms came onto the scene. With a population of increasingly diverse bacterial life, the stage was set for more life to form. There is compelling evidence that mitochondria and chloroplasts were once primitive bacterial cells. This evidence is described in the endosymbiotic theory. Symbiosis occurs when two different species benefit from living and working together. When one organism actually lives inside the other it's called endosymbiosis. The endosymbiotic theory describes how a large host cell and ingested bacteria could easily become dependent on one another for survival, resulting in a permanent relationship. Over millions of years of evolution, mitochondria and chloroplasts have become more specialized and today they cannot live outside the cell. Mitochondria and chloroplasts have striking similarities to bacteria cells. They have their own DNA, which is separate from the DNA found in the nucleus of the cell. And both organelles use their DNA to produce many proteins and enzymes required for their function. A double membrane surrounding both mitochondria and chloroplasts is further evidence that each was ingested by a primitive host. The two organelles also reproduce like bacteria, replicating their own DNA and directing their own division. Mitochondrial DNA (mtDNA) has a unique pattern of inheritance. It is passed down directly from mother to child, and it accumulates changes much more slowly than other types of DNA.

Because of its unique characteristics, mtDNA has provided important clues about evolutionary history. For example, differences in mtDNA are examined to estimate how closely related one species is to another. Conditions on Earth 4 billion years ago were very different than they are today. The atmosphere lacked oxygen, and an ozone layer did not yet protect Earth from harmful radiation. Heavy rains, lightning, and volcanic activity were common. Yet the earliest cells originated in this extreme environment. Extremophiles archaea still thrive in extreme habitats (Figure 1). Astrobiologists are now using archaea to study the origins of life on Earth and other planets. Because archaea inhabit places previously considered incompatible with life, they may provide clues that will improve our ability to detect extraterrestrial life. Interestingly, current research suggests archaea may be capable of space travel by meteorite. Such an event termed panspermia could have seeded life on Earth or elsewhere. The presence of archaea and bacteria changed Earth dramatically. They helped establish a stable atmosphere and produced oxygen in such quantities that eventually life forms could evolve that needed oxygen. The new atmospheric conditions calmed the weather so that the extremes were less severe. Life had created the conditions for new life to be formed. This process is one of the great wonders of nature.

RNA WAS THE FIRST GENETIC MOLECULE

Experiments in the 1960s showed that messenger RNA has the ability to store genetic information, while transfer and ribosomal RNA have the ability to translate genetic information into proteins. Experiments performed two decades later showed that some RNAs can even act as an enzyme to self-edit their own genetic code! These results raised two questions: 1) Why does RNA play so many roles in the flow of genetic information? 2) Why bother storing genetic information in DNA, if RNA alone could do the job? RNA has great capability as a genetic molecule; it once had to carry on hereditary processes on its own. It now seems certain that RNA was the first molecule of heredity, so it evolved all the essential methods for storing and expressing genetic information before DNA came onto the scene. However, single-stranded

RNA is rather unstable and is easily damaged by enzymes. By essentially doubling the existing RNA molecule, and using deoxyribose sugar instead of ribose, DNA evolved as a much more stable form to pass genetic information with accuracy.

How many species of animals are there

The answer to this question is - nobody knows! Scientists who study animal life are called zoologists. They have recorded 20,000 species of fish, 6,000 species of reptiles, 9,000 birds, 1,000 amphibians, and 15,000 species of mammals. And, although there are a million named species of insects, scientists estimate that there could be another million waiting to be discovered and named! The tragedy is that man is wiping out species so fast that children today will never have the opportunity of seeing many of those that are still living as they read this book. By the time they are grown up, many more species will be extinct. The destruction of the Amazon rain forests, for instance, which is taking place now, will wipe out thousands of species of animal life that man has not yet even identified.

How many kinds of trees are there?

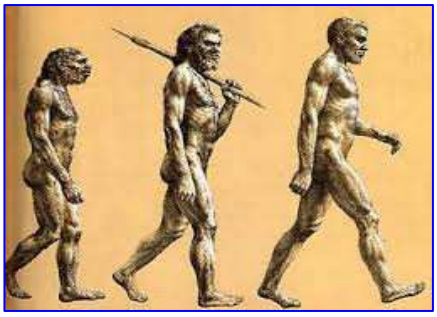
There are many types of trees out there. In North America, there are over 700 different types. In the world, there are around 100,000 type of trees, with a lot of them being endangered.

Fossil evidence

From skeletons to teeth, early human fossils have been found of more than 6,000 individuals. With the rapid pace of new discoveries every year, this impressive sample means that even though some early human species are only represented by one or a few fossils, others are represented by thousands of fossils. From them, we can understand things like:

- ✓ How well adapted an early human species was for walking upright
- ✓ How well adapted an early human species was for living in hot, tropical habitats or cold, temperate environments.
- ✓ The difference between male and female body size, which correlates to aspects of social behavior.

How quickly or slowly children of early human species grew up. While people used to think that there was a single line of human species, with one evolving after the other in an inevitable march towards modern humans, we now knows this is not the case. Like most other mammals, we are part of a large and diverse family tree. Fossil discoveries show that the human family tree has many more branches and deeper roots than we knew about even a couple of decades ago. In fact, the number of branches our evolutionary tree, and also the length of time, has nearly doubled since the famed 'Lucy' fossil skeleton was discovered in 1974! There were periods in the past when three or four early human species lived at the same time, even in the same place. We – *Homo sapiens* – are now the sole surviving species in this once diverse family tree. While the existence of a human evolutionary family tree is not in question, its size and shape - the number



of branches representing different genera and species, and the connections among them – are much debated by researchers and further confounded by a fossil record that only offers fragmented look at the ancient past. The debates are sometimes perceived as uncertainty about evolution, but that is far from the case. The debates concern the precise evolutionary relationships - essentially, 'who is related to whom, and how.' Click here to explore information about different early human species.

Source: http://humanorigins.si.edu/evidence/human-fossils

E. coli: A "MODEL ORGANISM"

In the late 1800's, the German pediatrician and bacteriologist, Theodor Escherich, was dismayed by the fact that many babies were dying of diarrhea. Because he believed in the "germ theory of disease," he felt that an organism was the causative agent and set about trying to find it. These investigations led him to discover what he called Bacillus communis coli, a Gram negative, rod-shaped bacterium, that is found in the lower intestines of humans as well as other warm-blooded organisms. After his death, it was renamed in his honor as Escherichia coli, commonly referred to an abbreviated as E. coli.1 Escherichia coli has become a "model organism" for studying many of life's essential processes. A model organism is a species that has been widely studied, usually because it is easy to maintain and breed in a laboratory setting and has particular experimental advantages. Due to its rapid growth rate, simple nutritional requirements, well established genetics and completed genomic sequence, more is now known about E. coli than any other living organism. It is currently the most widely-used organism in molecular genetics and many Nobel prizes have been awarded for studies based upon E. coli. E. coli has a cell division rate of about once every 30 minutes, enabling rapid adaptation to the environment. This rapid division

rate has facilitated a long term evolutionary experiment conducted in the lab. Beginning in 1988, Richard Lenski has tracked genetic changes in 12 nearly identical populations of E. coli, reaching 50,000 generations on February 24th of this year. Lenski's study is being conducted with a strain of E. coli that reproduces asexually to facilitate a better understanding of random genetic mutations. There are, however, strains of E. coli that reproduce sexually and can transfer DNA from one cell to another.

ANTIBIOTIC RESISTANCE BECOME A MATTER OF THOUGHT

ANTIBIOTIC resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth; in other words, the bacteria are "resistant" and continue to multiply in the presence of therapeutic levels of an antibiotic. Some bacteria are naturally resistant to certain types of antibiotics. However, bacteria may also become resistant in two ways: 1) by a genetic mutation or 2) by acquiring resistance from another bacterium. Mutations, rare spontaneous changes of the bacteria's genetic material, are thought to occur in about one in one million to one in ten million cells. Different genetic mutations yield different types of resistance. Some mutations enable the bacteria to produce potent chemicals (enzymes) that inactivate antibiotics, while other mutations eliminate the cell target that the antibiotic attacks. Still others close up the entry ports that allow antibiotics into the cell, and others manufacture pumping mechanisms that export the antibiotic back outside so it never reaches its target. Bacteria can acquire antibiotic resistance genes from other bacteria in several ways. By undergoing a simple mating process called "conjugation," bacteria can

Fig: Red Blood Cell

transfer genetic material, including genes encoding resistance to antibiotics (found on plasmids and transposons) from one bacterium to another. Viruses are another mechanism for passing resistance traits between bacteria. The resistance traits from one bacterium are packaged into the head portion of the virus. The virus then injects the resistance traits into any new bacteria it attacks. Bacteria also have the ability to acquire naked, "free" DNA from their environment. Any bacteria that acquire resistance genes, whether by spontaneous mutation or genetic exchange with other bacteria, have the ability to resist one or more antibiotics. Because bacteria can collect multiple resistance traits over time, they can become resistant to many different families of antibiotics.

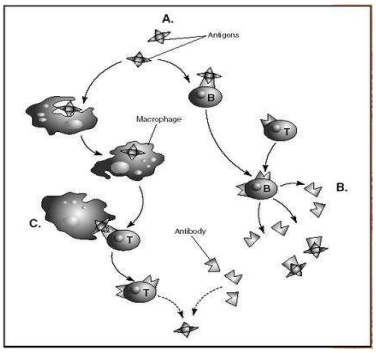
SMALL ERRORS IN PROTEINS CAN CAUSE DISEASE

Sometimes, an error in just one amino acid can cause diseases. Sickle cell disease, which most often affects those of African descent, is caused by a single error in the gene for hemoglobin, the oxygen-carrying protein in red blood cells. This error, or mutation, results in an incorrect amino acid at one position in the molecule. Hemoglobin molecules with this incorrect amino acid stick together and distort the normally smooth, lozenge-shaped red blood cells into jagged sickle shapes. The most common symptom of the disease is unpredictable pain in any body organ or joint, caused when the distorted blood cells jam together, unable to pass through small blood vessels. These blockages prevent oxygen-carrying blood from getting to organs and tissues. The frequency, duration, and severity of this pain vary greatly between individuals. The disease affects about 1 in every 500

Figure: Suckled Blood Cell

African Americans, and 1 in 12 carry the trait and can pass it on to their children, but do not have the disease themselves. Another disease caused by a defect in one amino acid is cystic fibrosis. This disease is most common in those of northern European descent, affecting about 1 in 2,500 Caucasians in the United States. Another 1 in 25 or 30 are carriers. The disease is caused when a protein called CFTR is incorrectly folded. This misfolding is usually caused by the deletion of a single amino acid in CFTR. The function of CFTR, which stands for cystic fibrosis transmembrane conductance regulator, is to allow chloride ions (a component of table salt) to pass through the outer membranes of cells. When this function is disrupted in cystic fibrosis, glands that produce sweat and mucus are most affected. Thick, sticky mucus builds up in the lungs and digestive organs, causing malnutrition, poor growth, frequent respiratory infections, and difficulties breathing. Those with the disorder usually die from lung disease around the age of 35.

How vaccine works



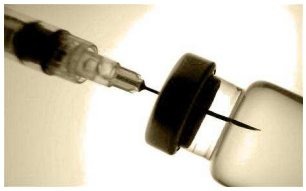
A Vaccine containing antigens are introduced into the body, stimulating the immune system response by instructing B cells, with assistance from T cells, to produce antibodies. B. Antibodies are produced to fight the weakened or dead viruses in the vaccine. The immune system prepares to destroy real and stronger viruses in the future. C. When new antigens enter the body, white blood cells called macrophages engulf them; process the information contained in the antigens, and sends it to the T cells so that an immune system response can be mobilized.

NOVEL VACCINE APPROACHES TO PREVENT DISEASE

VACCINE AGAINST HUMAN CYTOMEGALOVIRUS (CMV):

CMV is a type of herpes virus that is spread through close contact with the saliva, urine or other body fluids of a person infected with the virus. Most CMV infections are not diagnosed because the virus the virus -- a condition that is of most concern among pregnant women, who are at risk for transmitting the virus to the fetus. Children who are congenitally infected with CMV may have cognitive and other developmental disabilities; including hearing loss and blindness.CMV is the most common viral cause of congenital defects in the U.S. About 1 in 150 children in the U.S. is born with congenital CMV. While typically causes few, if any, symptoms. As a result, most people infected with CMV are unaware that they harbor most of these children will not develop symptoms or problems, about 1 of every 5 children with congenital CMV infection -- a total of 5,000 children each year -- will develop hearing loss or developmental disabilities due to the infection, according to the U.S. Centers for Disease Control and Prevention (CDC).CMV also can infect animals, including rodents and rhesus macaque monkeys and other non-human primates. However, the CMV species found in animals differs from human CMV and has not been reported to cause human disease.

CANCER VACCINE APPROACHES:



Cancer vaccines are medicines that stimulate or restore the immune system's ability to fight an existing cancer by strengthening the body's natural defenses against the cancer cells. Cancer vaccines are similar to traditional vaccines, which help prevent infectious diseases, such as

polio or measles, which protect the body against infection. Developing effective cancer treatment vaccines requires a detailed understanding of how immune system cells and cancer cells interact. The immune system often does not "see" cancer cells as dangerous or foreign, as it generally does with microbes. Therefore, the immune system does not mount a strong attack against the cancer cells not to mention cancer stem cells. Both cancer vaccines and traditional vaccines are based on antigens that are carried by foreign agents and that are relatively easy for the immune system to recognize as an "outsider." Several factors may make it difficult for the immune system to target growing cancers for destruction. Most important, cancer cells carry normal self-antigens (self-markers) in addition to specific cancer-associated antigens. Furthermore, cancer cells sometimes undergo genetic changes that may lead to the loss of cancer-associated antigens. Finally, cancer cells can produce chemical messages that suppress anticancer immune responses by cells known as killer T cells which play a central role in cell-mediated immunity. As a result, even when the immune system recognizes a growing cancer as a threat, the cancer may still escape a strong attack by the immune system. Producing effective treatment vaccines has proven quite challenging and to be effective, they must achieve two goals. First, like traditional vaccines, cancer vaccines must stimulate specific immune responses against the correct target. Second, the immune responses must be powerful enough to overcome the barriers that cancer cells use to protect themselves from attack by B cells and killer T cells. Recent advances in understanding how cancer cells escape recognition and attack by the immune system are now giving developers the knowledge required to design cancer treatment vaccines that can accomplish both goals. The rational approach to cancer vaccine design has primarily taken three different approaches to targeting cancer cells via (A) single antigens (B) tumor lysate vaccines that take entire proteins from tumor and create a very broad response, and (C) multiple antigens, targeting dominant antigens present on tumors.

VACCINE APPROACHES TO PREVENT TUBERCULOSIS:

Among infectious pathogens in the world and represents an important factor that sustain poverty in developing countries. Failure of the BCG vaccine to protect in endemic regions and increasing

problems with multi-drug-resistant TB calls for development of better vaccines to prevent reactivation of tuberculosis. It has been estimated that an effective post-exposure vaccine will prevent 30-40% of the TB cases. New vaccines should also prevent development of TB in HIV-infected individuals. Recent characterization of M. tuberculosis H37Rv by proteomic methods has revealed a large number of novel secreted proteins that should be investigated in mouse models for latent and slowly progressive TB. There is an important balance between control of infection and tissue destruction in TB, and M. tuberculosis has developed strategies to prevent immune-mediated sterilization. Central to this strategy is inhibition of apoptosis of macrophages. Development of novel vaccines should therefore take into consideration the effects on central markers to obtain a better picture of regulation of immunity, including FasL and Bcl-2 which are essential in regulation of apoptosis. Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is one of the main killers.

NEW HIV VACCINES:

There are several therapeutic vaccines in development. Approaching HIV in slightly different ways, all are designed to allow the body's immune system to at least fight the virus to a standstill, and perhaps even keep it at undetectable levels. Common to all treatments is giving the immune system some way to recognize HIV. The vaccines differ in the markers (called antigens) they use to flag HIV particles, and in how they are delivered to the body. Vacc-4x trains a person's immune system to recognize and fight a key protein that HIV relies on, called gp24. It also stimulates the production of white blood cells, which normally are killed by the virus. Early results show patients' viral loads coming down by a factor of three. Genetic Immunity, a U.S.-Hungarian company, is testing a vaccine called DermaVir. Rather than focusing on a single protein, DermaVir uses a tiny bit of HIV DNA (called plasmid DNA) to generate a set of 15 chemical markers that the body's T-cells can recognize. The idea is to maximize the number of ways the immune cells can "see" the virus. The vaccine is administered by rubbing the skin enough to irritate it. Cells called dendritic cells will pick up a nanoparticle containing the DNA and deliver it to the lymph nodes, where the infection-fighting T-cells are generated. The vaccine has been tested on about 70 patients so far and showed a 70 percent reduction in viral load,

according to Genetic Immunity's president, Dr. Julianna Lisziewicz. Another set of trials on patients is currently under way. Another approach is being taken by Gaithersburg, Md.-based Company VIRxSYS, which uses a genetically altered HIV virus to deliver the vaccine. The body doesn't recognize HIV easily, and thus won't mount an immune response to the very vehicle delivering the medicine, said Franck Lemiale, senior director of immunobiology at the company. To make sure that the T-cells will "see" many strains of HIV, the VIRxSYS vaccine uses proteins called Gag, Pol and Rev, which tend to be the same in all of variations of the HIV virus. The company said in July 2011 that a version of its vaccine tested in monkeys, called VRX1273, had not only brought the viral loads down to undetectable levels in body fluids, but in tissues as well. If that result can be duplicated in humans, it might mean that the vaccine is helping the body to.

GUIDELINES FOR PCR

The invention of the polymerase chain reaction (PCR) by K. Mullis and co-workers in 1985 revolutionized molecular biology and molecular medicine. Major research areas, such as biomarker discovery, gene regulation, and cancer research, are challenging today's PCR technologies with more demanding requirements. These include the need for increased throughput, higher assay sensitivity, and reliable data analysis. Assay development and evaluation, reproducibility of data, and time to result are still major problems encountered by researchers. PCR amplification is performed routinely and thousands of PCR protocols have been developed, yet researchers still encounter technical difficulties with PCR experiments and often fail to obtain specific amplification products. Although there are several different challenges (e.g., smearing, low yield, and nonspecific amplification), there are two main reasons for PCR failure or poor results: the specificity of the reaction and template secondary structure. PCR is both a thermodynamic and an enzymatic process. Successful real-time PCR requires

amplification and detection under optimal conditions and each reaction component can affect the result. The annealing step is critical for high PCR specificity. When primers anneal to the template with high specificity, this leads to high yields of specific PCR products and increases the sensitivity of the amplification reaction. However, due to the high primer concentration in the reaction, primers will also hybridize to non-complementary sequences with mismatches. If the primers anneal to the template sequence with low specificity, amplification of nonspecific PCR products and primer–dimmers may occur. Competition in the amplification reaction between these artifacts and the desired PCR product may reduce the yield of the specific product, thereby reducing the sensitivity and linear range of the real-time reaction. Low PCR specificity can significantly affect quantitative PCR particularly when using SYBR Green for detection. As SYBR Green binds to any double-stranded DNA sequence, primer–dimmers and other nonspecific PCR products will generate a fluorescent signal. This reduces the overall sensitivity of the assay and also leads to inaccurate quantification of the transcript of interest. Factors critical for high specificity in PCR includes primer design and the reaction chemistry used.

PCR CONDITIONS:

The primer and Mg^{2+} concentration in the PCR buffer and annealing temperature of the reaction may need to be optimized for each primer pair for efficient PCR. In addition, PCR efficiency can be improved by additives that promote DNA polymerase stability and processivity or increase hybridization stringency, and by using strategies that reduce nonspecific primer–template interactions (1). Use of high-purity reagents is also essential for successful PCR, especially for amplification of rare templates, for example, single copy genes in genomic DNA or pathogenic viral DNA sequences in genomic DNA isolated from an infected organism. Inclusion of control reactions is essential for monitoring the success of PCR reactions. Wherever possible, a positive control should be included to check that the PCR conditions used can successfully amplify the target sequence. As PCR is extremely sensitive, requiring only a few copies of target template, a negative control containing no template DNA should always be included to ensure that the solutions used for PCR have not become contaminated with the

template DNA. PCR setup should be performed in a separate area from PCR analysis to ensure that reagents used for PCR do not become contaminated with PCR products. Similarly, pipets used for analysis of PCR products should never be used for setting up PCR.

MICROBES AND BIOFUELS

Biofuels are made from living things or the waste that they produce. One of the most common biofuels, ethanol, is produced from plants. The plant material used is the edible part of the plant such as sugar cane (Brazil) and sugar beet (France) or corn kernels (USA) because it can easily be broken down to sugar (glucose). The sugar can then be fermented (broken down) to ethanol by microbes such as the yeast *Saccharomyces cerevisiae.* Not only is it expensive to convert edible plant material into ethanol; ethical issues are also involved. It has been argued that we shouldn't grow food stuffs for fuel when people in some developing countries don't have enough to eat. There is a worry that Brazil will remove large sections of their rainforest to produce sugar cane. This is an issue because the trees in the rainforest use up huge amounts of carbon dioxide while carrying out photosynthesis. As a result biofuels from food stuffs such as sugar cane are unlikely to provide a long term solution as a replacement to fossil fuels.

NEW WAYS OF PRODUCING BIOFUELS:

Scientists are investigating the use of cellulose to produce ethanol. The ethanol produced from cellulose is exactly the same as the ethanol that is created from edible plant parts. Cellulose ethanol is produced from lignocellulose which is a mixture of lignin, hemicelluloses and cellulose. These three materials make up the plant cell wall. The lignin is the glue that holds the cellulose fibers together and gives the plant its rigidity. The lignocellulose is the part of the plant that remains undigested by humans and most animals i.e. it is a non-foodstuff e.g. stalks, sawdust and wood chip. There is a huge amount of non-edible plant waste to recycle. Scientists have

turned to their attention to microbes to see if they can find any that are capable of converting the cellulose and even hemicellulose in lignocellulose into ethanol. The remaining lignin by-product can be burned to produce energy. They have looked in the strangest of places from termites' stomachs to the soil surrounding volcanoes. What they have found is a range of very different microbes that all have one thing in common - they produce a group of enzymes called cellulase. An archaeon *Sulfolobus solfataricus* lives in volcanic pools near Mount Vesuvius in Italy. It produces cellulase. Researchers are looking at ways of genetically modifying this microbe to see if they can get it to improve its performance and produce more cellulase. In the future *S. solfataricus* may be used to produce biofuel. Another common wood digester is the fungus *Trichoderma reesei*. It is found in nearly all soils and secretes huge quantities of cellulase. The fungus was originally discovered by the United States army during the Second World War. It was responsible for breaking down the cellulose in the soldiers' canvas tents and uniforms which meant they became very holey. It was known as 'jungle rot'. A company in Canada has harnessed the microbes' ability to convert straw into glucose. The company genetically modified the fungus so that it produces even larger quantities of cellulase. A staggering 75 % of the straw fibre is converted into sugar. The left over woody matter, lignin, is dried and then pressed into burnable cakes. The glucose is then fermented with yeast to produce the biofuel ethanol. Could algae be a realistic alternative to fossil fuels? Algae carry out photosynthesis. They use the energy from sunlight to convert carbon dioxide into sugar, which they then metabolize into lipids. Algae lipids can be processed into biodiesel while algae carbohydrates can be processed into bioethanol. This works well in the laboratory in small-scale bioreactors. Scientists are investigating if it is practical to grow algae on a large enough scale to see if they may be the biofuel producers of the future. Microbes could well be the key to powering cars in an environmentally sound way and in the not too distant future we could all be filling up at the pump with microbial-based fuel.

WHAT ARE GMOS?

GMOs, or "genetically modified organisms," are plants or animals that have been genetically engineered with DNA from bacteria, viruses or other plants and animals. These experimental combinations of genes from different species cannot occur in nature or in traditional crossbreeding. Virtually all commercial GMOs are engineered to withstand direct application of herbicide and/or to produce an insecticide. Despite biotech industry promises, none of the GMO traits currently on the market offer increased yield, drought tolerance, enhanced nutrition, or any other consumer benefit. Meanwhile, a growing body of evidence connects GMOs with health problems, environmental damage and violation of farmers' and consumers' rights.

ARE GMOS SAFE?

Most developed nations do not consider GMOs to be safe. In more than 60 countries around the world, including Australia, Japan, and all of the countries in the European Union, there are significant restrictions or outright bans on the production and sale of GMOs. In the U.S., the government has approved GMOs based on studies conducted by the same corporations that created them and profit from their sale. Increasingly, Americans are taking matters into their own hands and choosing to opt out of the GMO experiment.

ARE GMOS LABELED?

Unfortunately, even though polls consistently show that a significant majority of Americans want to know if the food they're purchasing contains GMOs, the powerful biotech lobby has succeeded in keeping this information from the public. In the absence of mandatory labeling, the Non-GMO Project was created to give consumers the informed choice they deserve.

THE FETUS IS AN ALLOGRAFT THAT IS TOLERATED REPEATEDLY

All of the transplants discussed so far are artefacts of modern medical technology. However, one tissue that is repeatedly grafted and repeatedly tolerated is the mammalian fetus. The fetus carries paternal MHC and minor H antigens that differ from those of the mother and yet a mother can successfully bear many children expressing the same nonself MHC proteins derived from the father. The mysterious lack of rejection of the fetus has puzzled generations of reproductive immunologists and no comprehensive explanation has yet emerged. One problem is that acceptance of the fetal allograft is so much the norm that it is difficult to study the mechanism that prevents rejection; if the mechanism for rejecting the fetus is rarely activated, how can one analyze the mechanisms that control it? The fetus is an allograft that is not rejected. Although the fetus carries MHC molecules derived from the father, and other foreign antigens, it is not rejected. Even when the mother bears several children to the same father, no sign of immunological rejection various hypotheses have been advanced to account for the tolerance shown to the fetus. It has been proposed that the fetus is simply not recognized as foreign. There is evidence against this hypothesis, as women who have borne several children usually make antibodies directed against the father's MHC proteins; indeed, this is the best source of antibodies for human MHC typing. However, the placenta, which is a fetus-derived tissue, seems to sequester the fetus away from the mother's T cells. The outer layer of the placenta, the interface between fetal and maternal tissues, is the trophoblast. This does not express classical MHC class I and class II proteins, making it resistant to recognition and attack by maternal T cells. Tissues lacking class I expression are, however, vulnerable to attack by NK cells (see Chapter 10). The trophoblast might be protected from attack by NK cells by expression of a non classical and minimally polymorphic HLA class I molecule—HLA-G. This protein has been shown to bind to the two major inhibitory NK receptors, KIR1 and KIR2, and to inhibit NK killing. The placenta may also sequester the fetus from the mother's T cells by an active mechanism of nutrient

depletion. The enzyme indoleamine 2, 3-dioxygenase (IDO) is expressed at a high level by cells at the maternal-fetal interface. This enzyme catabolizes, and thereby depletes, the essential amino acid tryptophan at this site. T cells starved of tryptophan show reduced responsiveness. Inhibition of IDO in pregnant mice, using the inhibitor 1-methyltryptophan, causes rapid rejection ofallogeneic but not syngeneic fetuses. This supports the hypothesis that maternal T cells, alloreactive to paternal MHC proteins, may be held in check in the placenta by tryptophan depletion. It is likely that fetal tolerance is a multifactorial process. The trophoblast does not act as an absolute barrier between mother and fetus, and fetal blood cells can cross the placenta and be detected in the maternal circulation, albeit in very low numbers. There is direct evidence from experiments in mice for specific T-cell tolerance against paternal MHC alloantigens. Pregnant female mice whose T cells bear a transgenic receptor specific for a paternal alloantigen showed reduced expression of this during pregnancy. These same mice lost the ability to control the growth of an experimental tumor bearing the same paternal MHC alloantigen. After pregnancy, tumor growth was controlled and the level of the T-cell receptor increased. This experiment demonstrates that the maternal immune system must have been exposed to paternal MHC alloantigens, and that the immune response to these antigens was temporarily suppressed. Yet another factor that might contribute to maternal tolerance of the fetus is the secretion of cytokines at the maternal-fetal interface. Both uterine epithelium and trophoblast secrete cytokines, including transforming growth factor (TGF)-β, IL-4, and IL-10. This cytokine pattern tends to suppress T_H1 responses (see Chapter 10). Induction or injection of cytokines such as interferon (IFN)-γ and IL-12, which promote T_H1 responses in experimental animals, promote fetal resorption, the equivalent of spontaneous abortion in humans. The fetus is thus tolerated for two main reasons: it occupies a site protected by a non immunogenic tissue barrier, and it promotes a local immunosuppressive response in the mother. We will see later that several sites in the body have these characteristics and allow prolonged acceptance of foreign tissue grafts. They are usually called immunologically privileged sites.

Page of Bangladesh

Polio-free Bangladesh

Along with other 10 Asian countries, Bangladesh was declared polio-free by the World Health Organization's (WHO) on Thursday. Bangladesh has been free of the disease over the past seven years, but it was only on Thursday that was formally recognized. WHO issues a certificate for a region when all of its countries remain polio-free for three consecutive years. India was the region's last country to report a case of polio in Jan 2011. Bangladesh could achieve the feat by a strategic mix of routine immunization campaigns, quality surveillance, regular observance of National Immunization Day (NIDs) and facilitation of strong multi-sectoral collaboration, said WHO. "We looked for even a remote house so that no one is missed out," Dr Jahan Afroz said on Thursday when Bangladesh was officially conferred polio-free status. Dr Afroz worked with the EPI that oversees national immunization for more than two decades since 1991. "Even we used to go to the remote places from Dhaka so that our workers are backed up and get encouraged," she told bdnews24.com. Bangladesh is one of the world leaders in vaccination. WHO says the country's success in protecting its people from diseases that can be prevented by vaccines has its roots in the late 1950s with campaigns against tuberculosis. The Extended Program on Immunization (EPI) began in 1979 when Bangladesh was gripped by such diseases. Polio came as an additional challenge to the already fragile healthcare system. More and more children were diagnosed with paralysis in late 1980s EPI boosted its routine immunization campaign. The routine immunization of oral polio vaccine started in 1985. Before that, only 2 percent of Bangladesh's children under 5 years old were immunized. But this number jumped to 60 percent within 10

years after routine immunization was introduced and steadily intensified. Polio cases came down drastically to one case in 2000 from 16 in 1996. Since 2000, there has been no case of polio in Bangladesh but in 2006 it appears to have made its way from neighboring India. After 6 months of extensive operations, which included 6 rounds of special immunization days and mop-ups, polio was, once again, eradicated by Nov 2006. Bangladesh has been enjoying polio-free status ever since. WHO says routine immunization had always been the foundation to popularize oral polio vaccines among parents in Bangladesh. The former EPI official, Dr Afroz said persuading parents was the key element of their strategy. “If you do not give the vaccine, your child can be paralyzed for the entire life” is what parents were told. “Sometimes it was difficult, but our workers could do it. They have that confidence,” she said. The confidence that motivated them increased as the campaign was given top priority by the state with prime ministers inaugurating immunization campaign. Dr Afroz said when a child is born the EPI workers would make sure it was given a polio vaccine. Apart from routine campaign, Bangladesh has to date observed 21 National Immunization Days for polio. WHO says the latest of such specialized campaign in Jan this year confirmed 100% coverage.

Breast cancer up in Bangladesh

A National Institute of Cancer Research and Hospital (NICRH) registry analysis showed 26 percent of their women patients between 2008 and 2010 suffered from breast cancer, followed by 21 percent afflicted by cervical cancer. 28 percent men suffering were down with lung cancer followed by 6 percent afflicted by esophagus, commonly known as gullet cancer. The National Institute is the only hospital in Bangladesh that has a cancer registry system.“We lack manpower, logistics and IT facilities that delay our publications and analysis,” Dr Md Habibullah Talukder, an associate professor of the institute while presenting his findings on cancer trends at a seminar. The hospital received 46110 patients at its outpatient department between 2008 and 2010. Of them, 27281 were confirmed afflicted by cancer. The report indicated that of those confirmed with cancer, 56.1 percent were male and 43.9 percent were female.. Dr Talukder said of the lung cancer male patients aged above 10, about 78.4 percent have a history of smoking, while 63.9

percent were current smokers. Of the female cancer patients, 3.5 percent had a history of smoking, while 3.3 were current smokers. “But we are concerned because breast cancer is striking more women now than before,” Talukder said, blaming it 'lifestyle changes.' “Globally breast cancer is the most prevalent form of cancer afflicting women, but in Bangladesh, more women were previously suffering from cervical cancer. But this new report showed that breast cancer cases have risen every year and now topped the list among women,” Dr Talukder said. He said delayed marriage, tendency to take babies lately and not to breastfeed kids might be responsible for more breast cancer cases. He, however, suggested nationwide cancer registry system “to assess the trends of cancer, especially data on which the population group suffer from which type of cancer, and other relevant information to address the menace”.

How organisms transferred

Transmission is the passing of a communicable disease from an infected host individual or group to a conspecific individual or group by one or more of the following means: droplet contact, direct physical contact, indirect physical contact, airborne transmission, and fecal-oral transmission. Transmission can also be indirect, via another organism. Indirect transmission could involve zoo noses or, more typically, larger pathogens like macro parasites with more complex life cycles. Disease can be directly transmitted in two ways. The first is horizontal disease transmission – from one individual to another in the same generation by either direct contact, or indirect contact air, such as via a cough or sneeze. The second is vertical disease transmission – passing a disease causing agent vertically from parent to offspring, such as through perinatal transmission. Pathogens must have a way to be transmitted from one host to another to ensure their species' survival. Infectious agents are generally specialized for a particular method of transmission. For example, a virus or bacteria that causes its host to develop coughing and sneezing symptoms has a great survival advantage – it is much more likely to be ejected from one host and carried to another. This is also the reason that many microorganisms cause diarrhea. The respiratory route is a typical mode of transmission among many infectious agents. If an infected person coughs or sneezes on another person, the microorganisms, suspended in warm, moist droplets, may enter the body through the nose, mouth, or eye surfaces. Diseases that are commonly spread by coughing or sneezing include: bacterial meningitis and chickenpox. When viruses are shed by an infected person through coughing or sneezing into the air, the mucus coating on the virus starts to evaporate. Once this mucus shell evaporates the remaining virion is called a droplet nucleus or quanta. The mucus evaporation rate is determined by the temperature and humidity inside the room. The

lower the humidity, the quicker the mucus shell evaporates thus allowing thedroplet nuclei to stay airborne and not drop to the ground. The low indoor humidity levels in wintertime buildings ensure that higher levels of droplet nuclei will survive: droplet nuclei are so microscopic that they are able to stay airborne indefinitely on the air currents present within indoor spaces. When an infected person coughs or sneezes, a percentage of their viruses will become droplet nuclei. If these droplet nuclei gain access to the eyes, nose, or mouth of an uninfected person (known as a susceptible) – either directly, or indirectly by touching a contaminated surface – then the droplet nuclei may penetrate into the deep recesses of their lungs. Viral diseases that are commonly spread by coughing or sneezing droplet nuclei include the common cold and influenza. Direct fecal-oral transmission is rare for humans at least. More common are the indirect routes: foodstuffs or water become contaminated and the people who eat and drink them become infected. This is the typical mode of transmission for infectious agents such as cholera, hepatitis A, and polio. Sexual transmission refers to any disease that can be caught during sexual activity with another person, including vaginal or anal sex or (less commonly) through oral sex. Transmission is either directly between surfaces in contact during intercourse or from secretions which carry infectious agents that get into the partner's blood stream through tiny tears in the penis, vagina, or rectum. Some diseases transmissible by the sexual route include: HIV/AIDS and Chlamydia. Sexually transmitted diseases such as HIV and Hepatitis B are thought to not normally be transmitted through mouth-to-mouth contact, although it is possible to transmit some STDs between the genitals and the mouth during oral sex. In the case of HIV this possibility has been established. It is also responsible for the increased incidence of herpes simplex virus 1 (which is usually responsible for oral infections) in genital infections and the increased incidence of the type 2 virus (more common genitally) in oral infections. Diseases that can be transmitted by direct contact are called contagious. These diseases can also be transmitted by sharing a towel (where the towel is rubbed vigorously on both bodies) or items of clothing in close contact with the body (socks, for example) if they are not washed thoroughly between uses.

6 GREAT THINGS MICROBES DO FOR US

1. MICROBES PLAY DEFENSE

The oodles of microbes that live on and inside us protect us from pathogens simply by taking up space. By occupying spots where nasties could get access to and thrive, good microbes keep us healthy. As Eisen explains, “It’s sort of like how having a nice ground cover around your house can prevent weeds from taking over.”

2. MICROBES BOOST THE IMMUNE SYSTEM

Researchers at Loyola University demonstrated in a 2010 study how Bacillus, a rod-shaped bacteria found in the digestive tract, bind to immune system cells and stimulate them to divide and reproduce. The research suggests that, years down the road, those with weakened immune systems could be treated by introducing these bacterial spores into the system. These microbes could potentially even help the body fight cancerous tumors.

3. MICROBES PROTECT US FROM AUTO-IMMUNE DISEASES

In his TED Talk Eisen describes being diagnosed with Type 1 Diabetes as a teenager after “slowly wasting away until I looked like a famine victim with an unquenchable thirst.” Because microbes help train the immune system, if the micro biome is thrown out of whack, it can alter the body’s ability to differentiate between itself and foreign invaders. Recent research into Type 1 Diabetes reveals that a disturbance in the microbial community could trigger the disease, in which the body kills cells that produce insulin. In a 2009 study, researchers at Cornell University showed that introducing a benign strain of E. coli into diabetic mice set off a domino effect that led them to produce insulin. The work suggests that, someday, microbial yogurt could replace

insulin shots for people with the disease. Microbial disturbances could be at the root of other auto-immune disorders too.

4. MICROBES KEEP US SLIM

Microbes play an important role in our body shape by helping us digest and ferment foods, as well as by producing chemicals that shape our metabolic rates. Eisen explains, “It seems that disturbances in our microbial community may be one of the factors leading to an increase in obesity.”

5. MICROBES DETOXIFY AND MAY EVEN FIGHT OFF STRESS

Just as humans breathe in oxygen and release carbon dioxide, microbes in and on us take in toxins and spare us their dangerous effects. A recent study also shows that people feeling intense stress has much less diverse bacterial communities in the gut, suggesting that there is a not-yet-understood interplay between microbes and stress responses.

6. MICROBES KEEP BABIES HEALTHY

Recent studies have shown that babies born via caesarean section have very different micro biomes than those born the old-fashioned way. Why? Because during the birthing process, babies are colonized with the microbes of their mother, especially substances that aid in the digestion of milk. According to Science News, babies born via C-section are more likely to develop allergies and asthma than children born

Source: http://blog.ted.com/2012/07/10/6-great-things-microbes-do-for-us/ginally

How BACTERIA TALK TO EACH OTHER

Bacteria communicate with one another using small chemical molecules that they release into the environment. These molecules travel from cell to cell and the bacteria have receptors on their surfaces that allow them to detect and respond to the buildup of the molecules. This process of cell-to-cell communication in bacteria is called "Quorum Sensing" and it allows bacteria to synchronize behavior on a population-wide scale. Bacterial behaviors controlled by quorum sensing are usually ones that are unproductive when undertaken by an individual bacterium acting alone but become effective when undertaken in unison by the group. For example, quorum sensing controls virulence, sporulation, and the exchange of DNA. Thus, quorum sensing is a mechanism that allows bacteria to function as multi-cellular organisms. Cell-to-cell communication in bacteria was likely one of the first steps in the evolution of higher organisms. Current biomedical research is focused on the development of novel anti-bacterial therapies aimed at interfering with quorum sensing. Such therapies could be used to control bacterial pathogenicity

Source: http://online.itp.ucsb.edu/plecture/bbassler11/

Stem cell therapy for autism

Cell Therapy Center EmCell offers stem cell treatment for autism. Stem cell therapy is a novel and effective approach to treating autism and is based on the unique ability of stem cells to influence metabolism, immune system and restore damaged cells and tissues. EmCell's experience in treating autism and the results achieved in patients prove the role of EmCell. Fetal stem cells (FSC) that we use in autism treatment positively affect all body organs and systems, and, first of all, this treatment targets the brain. In autism, areas of brain regulating memory, concentration, attention, speech etc. are damaged. Stem cell treatment improves blood and oxygen flow to the brain (improved perfusion), replaces damaged neurons and stimulates formation of the new arteries. After some time, FSC acquire properties of cells surrounding them and multiply into these cells, which results in white and gray matter restoration and, consequently, in subsidence of neurologic symptoms and improved intellectual capacity. It has been proven that mesenchymal stem cells improve immune system and terminate inflammation. CD34 stimulation helps formation of the new arteries in hypoxic tissues, thus they increase blood flow in the temporal lobe and other parts of the brain. We treat autism with meso- and ectodermal stem cells harvested from 5-8 week old fetuses, tested and certified by the state. These cells are pluripotent and, administered to a patient, differentiate into cell types needed by the body.

STEM CELL THERAPY TARGETS SEVERAL ASPECTS OF CONCERN:

- ✓ Immunity;
- ✓ Metabolism;
- ✓ Communication ability; and
- ✓ Learning capacity, memory, and thinking.

Improvement is reached through restoration of the lost (impaired) neuron connections and formation of the new neuron connections, speeding up brain reactions through improvement of synaptic transmission and development of the new neuron connections.

3 OF 3

TOP 3 ACCIDENTAL INVENTIONS:

- **Penicillin.**
- **Pacemaker.**
- **Mauve.**

TOP 3 UNIVERSITIES FOR MEDICINE:

- **Harvard University.**
- **University of Oxford.**
- **University of Cambridge.**

Source: http://www.topuniversities.com/courses/medicine/top-10-universities-medicine

3 SYMPTOMS OF STRESS:

- **Tension.**
- **Anxiety.**
- **Irritability.**

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