The Changeable Nature of AIDS Virus

David Bardell

David Bardell is a professor of biology at Kean College of New Jersey, Union, NJ 07083. He has a B.A. in zoology from Hunter College of the City University of New York and a M.S. in zoology and a Ph.D. in microbiology from the University of New Hampshire. The author of numerous research and teaching articles, Bardell did postdoctoral research in microbiology at Harvard University School of Public Health. He has received research grants in virology from the American Philosophical Society and the National Science Foundation and a teaching grant from NSF.

At the present time there is no vaccine to prevent Acquired Immune Deficiency Syndrome (AIDS), a virus-induced disease. Furthermore, there is no cure for those who become ill with the disease. Because of the enormity of the problems in developing either a vaccine or curative agents, preventing transmission of the virus is the only means now available for controlling the spread of AIDS. It is likely to be the only means of combating the disease for a number of years to come.

To prevent the spread of the virus from one person to another the public must be educated to the virus' modes of transmission and to the dangers of the disease if it occurs. The public should also be informed of the problems confronting scientific efforts to overcome AIDS. This is necessary because the public at large tends to think that modern science has the know-how to quickly solve medical problems, especially in view of its many successes in the present century. There is a complacency built on the false assumption that the problem is not as great as it is made out to be, and that medicinal products that are effective against the disease will quickly become available. With respect to the successes of science during this century, not one "miracle" or "wonder" drug came into existence in a short period of time. Instead, they resulted from much investigative effort over many years. The purpose of the present article is to discuss a major problem confronting investigators working on the development of a vaccine against AIDS: evolution of variants of the causative virus.

The immune system of animals plays a major role in protecting them from infections by microorganisms, which are ever present and numerous in the environment. That this is so can be demonstrated in several ways, but is most easily observed after death. Without functioning defense mechanisms, the animal body is rapidly invaded by microorganisms that bring about putrefaction and other forms of decomposition of the body.

All animals, invertebrates and vertebrates, have evolved defense mechanisms against microorganisms, with birds and mammals having the most highly developed immune systems in the animal kingdom. The mammals' immune system consists of two parts—humoral and cell-mediated. The humoral part involves B-lymphocytes and the production of antibodies, which are released from cells into the blood and other body fluids, after which the antibodies bring about destruction or some other form of inactivation of antigens. The cell-mediated part involves T-lymphocytes and sensitized T-lymphocytes, cells that directly interact with and destroy antigens.

An antigen is an entity that elicits an immune response. Immune system cells come into contact with the surface of invading micrroorganisms, and antigens are part of their surface structure. Antigens most commonly are proteins or polysaccharide forms of carbohydrates. The cells of the immune system can distinguish between proteins and polysaccharides of the body and those which are foreign to the body. Therefore, the body does not usually produce antibodies or sensitized T-lymphocytes to its own proteins and polysaccharides.

In addition to T-lymphocytes that are directly involved in destruction of antigens, there are other kinds of T-lymphocytes that have a regulatory effect on the immune system. T-helper lymphocytes stimulate both humoral and cell-mediated parts of the immune system to respond more effectively to antigens. There are also T-suppressor lymphocytes that inhibit the immune system response after elimination of the antigen.

AIDS is caused by human immunodeficiency virus, which infects and destroys T-helper lymphocytes, impairing the immune system of the infected person. Originally the virus was known by several names, reflecting disagreement among investigators over an appropriate name. Although the virus now has a universally accepted name, it is usually referred to as AIDS virus and will be herein.

The immune system of a healthy animal functions at all times to allow the animal to cope with the numerous microorganisms in its surroundings. Most people believe the immune system only functions when a person is actually ill with an infectious disease. People do not realize that humans beings often become infected with microorganisms, but do not become ill since the immune system overcomes the microorganisms before they are able to cause symptoms of disease. Such infections are called subclinical infections.

With such a highly developed defense mechanism as the immune system, you might wonder why the body is ever subjected to infectious diseases by microorganisms. However, just as the animal body has evolved mechanisms for preventing invasion by microorganisms, some microorganisms have evolved mechanisms for combating the body's defense mechanisms.

An example of a microorganism being able to combat the body's immune system can be seen in the disease known as trypanosomiasis, commonly called sleeping sickness. The disease is caused by a protozoan microorganism of the genus *Trypanosoma* and is transmitted by the tsetse fly, hence the disease occurs in tropical areas inhabited by the fly. The disease is characterized by fever, inflammation of the lymph nodes and involvement of the brain and spinal cord, leading to lethargy and somnolence. Coma and death are the final outcome. It is a disease not only of human beings, but of domestic animals. Therefore, it is not only a hazard to human health, but also to animal sources of food for human beings.

A vaccine for the prevention of trypanosomiasis in humans and domestic animals would be highly desirable. However, despite considerable effort for several decades, attempts to produce a vaccine have been thwarted by antigenic variation of the trypanosomes. Although infected persons produce protective antibodies to the microorganisms, a new population of microorganisms that are antigenically different arise in the body. The antibodies are not effective against these. The immune response is highly specific, and antibodies and sensitized T-lymphocytes only respond to those antigens that induced their formation. Antigenic change in trypanosomes occurs as a result of surface proteins being replaced by antigenically different proteins. An analysis of the genetics of the process indicates that the trypanosomes possess a number of genes for surface protein and that genetic variation occurs as a result of random gene rearrangement (Tizard 1984). As the immune system responds to the variant, the variant is changing to another variant, and so the cycle of events goes on.

A situation similar to that observed with trypanosomes occurs with AIDS virus. However, antigenic variation of AIDS virus is not the result of gene shuffling, but is due to mutations which give rise to different strains of the virus.

AIDS virus is spherical. Its genetic material, ribonucleic acid (RNA), is located in a central position and is surrounded and protected by two layers of protein. Each layer is made of a different protein. The layers of protein are, in turn, surrounded by an envelope made up of lipid, protein and carbohydrate. In the center, together with the RNA, are molecules of an enzyme called reverse transcriptase. This enzyme has an essential role after the virus has infected a cell (Gallo 1987).

For a virus to infect a cell, there must be physical contact with the cell and also an affinity between the virus and the cell. This is why a specific virus cannot infect all kinds of cells and why a specific kind of cell cannot be infected by all kinds of viruses. With AIDS virus, there is an attraction between certain sites on the cell membrane of T-helper lymphocytes and parts of the viral envelope consisting of glycoprotein (a combination of protein and carbohydrate). After contact with a T-helper lymphocyte, the virus enters the cell, the protective protein layers around the RNA are removed and the genetic material is then able to express itself. The strand of RNA serves as a template for the formation of a complementary strand of deoxyribonucleic acid (DNA), and this requires the enzyme reverse transcriptase. During replication a virus uses the biochemical machinery of its host cell. However, the cells of animals and plants and microorganisms never synthesize DNA using RNA as a template. DNA is synthesized using DNA as a template.

It is for this reason that AIDS virus must have a gene for reverse transcriptase. With the gene they can induce the host cell to produce the enzyme for incorporation into the virus. When the complementary strand of DNA has been produced, it serves as a template for the production of a complementary strand of DNA, giving rise to double-stranded DNA. The genetic material of animals and plants and microorganisms (with the exception of some viruses) consists of double-stranded DNA. Synthesizing DNA using DNA as a template requires an enzyme called DNA polymerase. But since cells naturally do this, they have the enzyme, and at this stage the virus uses what is already available in the host cell. The double-stranded viral DNA enters the nucleus of the cell and becomes integrated into the cell's DNA. Thus, the viral genes become part of the host cell's genes.

When a virus has incorporated its genes into the chromosomal DNA of a cell it is called a provirus. The AIDS provirus usually remains latent until the host cell is stimulated during subsequent infection by a microorganism, for example by herpes simplex

virus type 1. The AIDS virus then reproduces itself giving rise to numerous progeny viruses; this process destroys the host cell. The progeny viruses then infect other T-helper lymphocytes, which eventually results in the destruction of more cells and consequent increasing impairment of the immune system.

Viruses that have RNA as their genetic material generally mutate at a greater rate than viruses having DNA genes. One reason for this is that RNA is less stable than DNA (Steinhauer & Holland 1987).

AIDS virus is classified into a group called the Retroviridae. The major characteristic of this group is the members' ability to synthesize DNA using RNA as a template. Studies have revealed that mutations can be generated by several mechanisms during retrovirus replication (Steinhauer & Holland 1987).

Point mutations occur at a high frequency during reverse transcription, the synthesis of DNA using RNA as a template. DNA and RNA consist of component molecules called nucleotides. A gene comprises a certain sequence of nucleotides that dictates a particular characteristic of an organism. A mutation is a change in the sequence of nucleotides of a gene. A point mutation is the substitution of one kind of nucleotide for another. This can result in changes in a characteristic, for example a change in the antigenic nature of a protein. In addition to substitution, a nucleotide may be added or deleted from a nucleotide sequence, and either case can result in a heritable change in a virus.

The mechanism of substitution, insertion and deletion of nucleotides is commonly due to errors during polymerization of nucleotides. There are 9,500 nucleotides making up the genetic material of AIDS virus (Gallo 1987). Not only does the length of the single linear strand of nucleotides lend itself to the possibility for errors, but also the mode of retrovirus replication, since polymerization occurs three times during replication. The first instance occurs when DNA is produced using viral RNA as a template; the second occurs when the complementary strand of DNA is synthesized to give double-stranded DNA for incorporation into host cell chromosomes; and the third, when RNA is produced using incorporated DNA as a template. This RNA from the third polymerization is the genetic material that becomes enclosed in progeny viruses.

Mutant retroviruses may also arise by recombination. In this, part of the viral DNA is replaced by a sequence of nucleotides from other viral or cellular DNA (Steinhauer & Holland 1987). There are several ways in which this can happen, for example an exchange between unintegrated viral DNA and integrated proviral DNA, or between viral DNA and host cell DNA. Mutations brought about by recombination involving viral DNA are reflected in the RNA of progeny viruses.

There are many successful viral vaccines, but these successes have been achieved with antigenically stable viruses. A sobering fact with respect to vaccine development where antigenically unstable viruses are the target can be seen with influenza virus. Several decades of research has not led to a successful vaccine for general use. When persons receive influenza vaccine or have a natural infection with influenza virus, they produce protective antibodies to the virus. However, influenza virus mutates at a fast rate, and before long persons are infected with a mutant against which their antibodies are not effective. Consequently, there are major epidemics, or even pandemics, of influenza every few years. Another sobering fact of AIDS virus as compared with influenza virus is the greater mutation rate of AIDS virus.

According to Darwin's concept of evolution, variation exists between individual members of a species and nature selects those variants that are best able to survive. With natural selection of variants, new species could arise. Darwin's observations of animal species on the Galapagos Islands were a major factor giving rise to his conclusion that different species of organisms had not been created separately and thereafter had remained unchanged with passing time. He was of the opinion that the distinctly different but related species of animals found on the islands—the 13 species of finches, for example—were descended from a single species. He collected a massive amount of data to support his concept. His observations of domestic forms of animals and plants were of great importance as was the observation that, for breeding, humans selected individuals of a species with a characteristic considered advantageous for human purposes. Domestic organisms were therefore somewhat different from wild organisms. Although it would be a slower process than the artificial selection by humans, natural phenomena could also bring about the selection of variants (Darwin 1872). Darwin did not know what the mechanism of variation was; since his time it has been found to be mutation.

AIDS virus demonstrates well the basic tenets of Darwinian evolution: that nature selects those variants that are best able to survive. No doubt there are mutations of AIDS virus that alter surface proteins so that the mutants lack the ability to interact with cells. Such mutants, of course, do not survive since an intracellular environment is absolutely essential for virus replication. However, there are mutations which change surface proteins enough to affect the antigenicity of the virus, but not its ability to infect T-helper lymphocytes. When viruses replicate, one virus in a single replicative cycle gives rise to numerous progeny viruses. Such a mode of replication contributes to the possibility that a mutation to a variant form of the virus will appear, a mutation that

is beneficial, as it allows the variant AIDS virus to escape the immune response of an infected person.

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