The World Health Organization estimates that more than 500,000 children worldwide have AIDS, and that more than 1 million may be infected with HIV, the virus most scientists agree is the cause of AIDS. At ASU, researchers are studying how to prevent the spread of HIV from mother to child.

by Alana Mikkelsen

The statistics are sobering. The number of young victims is expected to jump to 10 million by the year 2000. Nearly one-third of those children will die before their fifth birthday. Most will not live beyond age nine.

Despite this impact and HIV’s continuing rampage through the heterosexual population, doctors and scientists still know little about how the virus is transmitted from mother to child, how to prevent the transfer, or how to most safely treat pregnant women who have AIDS or who are infected with HIV.

A team of scientists at Arizona State University is working to answer those questions and to characterize some of the dynamics surrounding mother-to-child transmission of the virus. Results from some of their work is shedding surprising new light on the possible promise and price of treating pregnant women with antiviral drugs, particularly Zidovudine or Zidovudine.

Zidovudine’s impact on women has become almost astounding. The statistics are sobering. AIDS is now the leading cause of death of females aged 25 to 44 in New York City. AIDS is the fastest-growing killer of women of childbearing age in the United States. AIDS is among the top four causes of death of the 25-44 female age group nationwide. AIDS is the ninth leading cause of death of children between the ages of one and four in the world.

Increasing numbers of women are taking antiviral drugs to combat HIV infection. But these drugs were tested primarily on gay men in the desperate effort to quell the epidemic in the early 1980s. There is little data on whether drug doses found safe in those groups are as effective in other groups of people.

In light of these knowledge gaps, AIDS activists, women’s groups, and the National Institutes of Health all are calling for more research on how HIV/AIDS and its treatments affect women and their babies.

Zidovudine is the most commonly prescribed anti-HIV drug. It is also called azidothymidine or zidovudine. To date, no one has detected any negative effect of Zidovudine on mothers or their unborn children.

This is where ASU researchers enter the story.

“Our work suggests that we might want to be a little cautious there, especially with early pregnancies,” says Bertram Jacobs, an ASU associate professor of microbiology. Jacobs works with ASU developmental biologists Robert McGaughey, David Capco, and a number of graduate students.

Zidovudine was approved as an AIDS treatment in 1987, and was authorized for use in pregnant women by the Food and Drug Administration in August 1994. In February 1995, officials at the federal Centers for Disease Control in Atlanta recommended that all pregnant women in the United States be tested for HIV and, if positive, that they be offered Zidovudine treatment. Both the CDC recommendation and the FDA approval were based on a study that showed Zidovudine treatment after 14 weeks of pregnancy could reduce HIV transmission from mother to infant by about two-thirds.

The study was part of a randomized, placebo-controlled clinical trial sponsored by the National Institutes of Health. The results, reported in a November 1994 edition of The New England Journal of Medicine, were so dramatic that the trial was stopped mid-stream to allow women receiving placebos to take advantage of the treatment. The results indicated that HIV-positive women gave birth to infected babies in 26 percent of untreated cases, while short-term Zidovudine therapy cut the risk to 8 percent.

Treatment was given to the mothers during the second and third trimesters of pregnancy, at delivery, and to the child for six weeks after birth. As a result, Zidovudine treatment was judged safe for both mothers and infants.

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But results of studies conducted by ASU scientists suggest that, in earlier stages of pregnancy, the drug might not be so harmless.
“It appears that embryo cells may be more sensitive to Azt than other cells,” says Robert McGaughey, a developmental biologist and Asu professor of zoology. McGaughey and fellow Asu embryologist David Capco conceived the experiments along with Jacobs, whose specialty is viruses.

Researchers in McGaughey’s laboratory harvested recently-fertilized mouse embryos, transferred them into plastic laboratory dishes, then watched how they developed when treated with increasing doses of Azt. Normally, the two-celled embryos would divide overnight into four-cells. After four days, they should round into a hollow ball of cells known as the blastocyst, which would be ready to implant into a womb.

But the researchers found that under the highest concentrations of Azt, almost all the embryos developed severe abnormalities or died. The effect was observed when the amounts of Azt used were analogous to drug concentrations used to treat people with Aids.

McGaughey worries that if Azt has the same effect on early human embryos, women given the drug may suffer abortions before they even know they have conceived. Or, if treated with Azt early in pregnancy, they may give birth to abnormal babies.

“The rule for mammalian embryo development is that the early stages are most susceptible to conditions resulting in cell death,” McGaughey explains.

The mouse embryos in the Asu experiments were treated with drugs just days after fertilization, which is before the embryos would have implanted themselves in the uterus or a pregnancy test would have turned up positive.

“If you damage a pre-implantation embryo, even subtly, you’re going to kill it,” McGaughey continues. “Whereas, if the embryo is perfectly healthy at the stage of implantation, you’re less likely to kill it, but more likely to cause developmental damage.”

**MONKEYWRENCHING CELL HIJACKERS**

Azt is a drug designed to weaken viruses, but it also can damage human tissue. Viruses, including Hiv, are peculiar particles. Viruses are little globs of genetic material wrapped in a protein packet. They cannot reproduce without the help of genetic machinery found within living cells. Viruses are true, tiny parasites—although most scientists do not consider them to be technically alive.

Most antiviral drugs work by disrupting compounds the virus needs to reproduce itself. In the case of Azt, that compound is a molecule called reverse transcriptase. Reverse transcriptase is present in all members of a viral family called retroviruses, which includes Hiv.

Retroviruses use reverse transcriptase like a dictation secretary putting spoken words into written form. The genetic instructions found in a retrovirus come in the form of Rna, as the blastocyst, which would be ready to implant into a womb.

Once its genetic instructions have been translated into Dna form, the virus goes about its task of hijacking the cell’s own reproductive machinery. The impostor’s instructions are read by the cell. Unwittingly, the cell has become a factory for making new copies of the virus. The cell works overtime producing new viral Rna and proteins until it can hold no more. Then the cell bursts, spewing thousands of new viral particles into the blood, particles that immediately go in search of new cells to infect continuing the cycle of virus production and cell death.

To make Dna from Rna, reverse transcriptase strings together thousands of genetic “letters,” or bases. Azt resembles one of those nucleosides, called thymidine. The drug actually competes with thymidine for a place in the growing Dna chain. If Azt wins, it disrupts the Dna building process and the virus has no way to make the cell understand its instructions. The cycle of cellular hijacking ends.

But Azt can disrupt the reproduction of normal cells as well, because normal cells use the same nucleosides, including thymidine, to make Dna from Dna before they replicate. The effects are most devastating to rapidly dividing cells such as red blood cells. Aids patients treated with Azt often suffer from severe anemia, which is caused by a depletion of red blood cells.

Results of the Asu researchers’ studies suggest that embryo cells, which also divide rapidly, are also likely targets.

Azt is not licensed for use in women less than 14 weeks pregnant, and most of the clinical studies showing its safety in expectant mothers have been carried out after that time. However, once a drug is available for one purpose, it generally can be given for others.

But if Azt damages early human embryos, as the Asu studies suggest it might, the effects will not have been obvious to date, McGaughey says. Women in their first trimester of pregnancy are likely to be unaware they have conceived, and if Azt causes spontaneous early abortions, it would not be surprising for them to go unnoticed, he adds.

Consequently, McGaughey suggests that future clinical trials should compare the birth rates of Hiv-positive women taking Azt to those of Hiv-positive women not taking the drug. The Asu scientist recommends that Hiv-positive women avoid becoming pregnant and that they be tested for pregnancy before making a decision about Hiv treatment. McGaughey also cautions women and doctors to carefully consider the risks of extending Azt application beyond the drug’s labeled use.

Despite these gloomy overtones, the Asu team’s discoveries also offer hope.

**THE POWER OF INTERFERON**

“Probably the most important observation of these studies was that if we included mouse interferon with Azt, the deleterious effect of Azt was to some extent reversed,” McGaughey explains.

Interferon is a natural chemical produced to combat infection. It is the body’s first line of defense against viruses. A synthetic form of the substance is being tested in Aids patients for anti-Hiv activity. The Asu researchers included interferon in their studies partly to observe its effects on early development. But no one expected interferon to overcome Azt damage.

The scientists certainly didn’t expect another of the results that they recorded. When embryos were cultured in interferon but not Azt, the scientists found that the embryos progressed “better” than embryos grown in the usual concoction of chemicals designed to nurture development in a laboratory dish.

“There’s never been any strong indication that interferon is involved in the development of mice or humans,” says Bertram Jacobs.

The Asu virologist has been studying interferon and its antiviral properties for almost 15 years. He says that the new findings point to all kinds of previously unexplored questions about the chemical’s role inside the womb. Embryologist McGaughey agrees.

“We feel we have evidence that there is a cellular interaction between interferon and these (mouse) embryo cells,” McGaughey says. “And our next question is: Do interferons function in early embryogenesis (to regulate) development?”
At right, an in vitro cultured mouse blastocyst imaged by fluorescence light microscopy. At this stage the developing embryo is a small ball of cells. The nuclei of the cells are made bright by a chemical dye that fluoresces and makes cell structure more apparent. Cells affected by AZT often show abnormalities by this point of their development.

Very little information is available on that topic, note Jacobs and Mcgaughey.

In a related direction, work by the ASU scientists also offers preliminary evidence that, with respect to AZT, interferon could eventually help the medical community solve a classical dilemma of giving drugs to pregnant women. The dilemma: treat the mother and risk unknown effects on the baby, or, avoid possible damage to the fetus at the cost of the mother’s health and, possibly, her life.

In the laboratory, interferon can destroy Hiv-infected cells and, with them, the virus. But in tests on live human beings, interferon generally has failed to deliver on that promise.

“Clearly, interferon has not been the magic bullet as an anti-Hiv agent that we would like,” Jacobs explains. “And nobody knows why that is.”

Scientists had hoped interferon might prove a more effective, less toxic drug than AZT, but current results suggest it is unlikely that interferon will reduce symptoms in AIDS patients. Still, interferon given to women in combination with AZT might have an altogether different effect.

HIV ACROSS THE PLACENTA If the results of the preliminary studies at ASU are extended and supported, it might one day be possible to give interferon to Hiv-infected women who are being treated with AZT, prevent possible AZT damage to any early embryos, and possibly even protect prenatal babies from infection with Hiv that is transmitted across the placenta. The placenta is the primary barrier between the blood of the mother and that of the growing fetus.

Scientists don’t know exactly how Hiv is transmitted from mother to child. The placenta is just as likely a doorway for the virus as two other possibilities: blood and body fluid exposure at birth, and breast-fed milk.

“The placenta is the perfect place for interferon to do its job,” says Scott Shors, who tested the influence of interferon on viral replication in the placenta while a doctoral candidate at ASU. Shors now works as a postdoctoral fellow at the National Institutes of Health.

“Depending on the study you believe, between 15 and 40 percent of mothers infected with Hiv do not give birth to infected babies,” Shors says. “This implies that the placenta is a viable barrier to Hiv infection, at least in some cases.”

Shors and his colleagues wondered if the effectiveness of the placenta could be boosted by interferon, which had been associated with the placenta in other studies.

“People knew that the placenta made interferon and set up what is called an ‘antiviral state.’ Such a state prevents viruses from replicating,” Shors says. “But nobody had ever tested its sensitivity to interferon before.”

Shors did just that. He took fresh placental cells, grew them in the laboratory, treated them with interferon, and then washed out the interferon.

Shors then infected the cells with three viruses unrelated to Hiv and counted how many new virus particles were shed by the infected cells. The interferon-treated cells gave rise to 100 times fewer viruses than untreated controls, implying that the treatment helped the placenta shut down viral replication.

Interferon normally destroys viruses indirectly by triggering infected cells to produce a host of chemicals that disrupt various internal processes, including those necessary for viral reproduction.

The barrage of chemicals causes the cells to undergo a sort of “programmed suicide,” Shors explains. When the cells die, they generally take their dependent viruses with them. The damage to the body is minimal, Shors says.

“The human body contains thousands of trillions of cells. You can afford to laugh off losing a few, as long as you kill the virus,” he adds.

Although results from tests with placental cells are promising, the researchers warn that they are preliminary and deserve follow-up for several reasons. First, the scientists used placenta taken after natural birth, when the cells are programmed to die. The researchers say that placental cells that protect the fetus at earlier stages of pregnancy may react to interferon very differently.
“It’s like working on a geriatric person and saying this drug works well on them, but we don’t know if it works well on children,” Shors explains. “There’s no reason to believe that it won’t, but we need to do the tests to be completely sure.”

Second, the Asu scientists did not test whether the interferon-treated placenta would block Hiv replication. They stress that that is a crucial next step, particularly since the mechanism and effectiveness of interferon action against Hiv remains a mystery.

“When a cell picks up the interferon signal, it makes as many as a dozen different proteins with possible antiviral effects,” Shors explains. “To date, it’s very unclear which of these proteins are important for stopping Hiv.”

Results of some studies, including the seemingly unsuccessful clinical trials with Aids patients, suggest that at least some strains of Hiv might be resistant to interferon. If that is so, Jacobs does not feel that studies on Hiv and interferon will be particularly fruitful.

For now, the Asu researchers intend to concentrate on prying a little wider the door they seem to have cracked open into interferon’s role in cell development. McGaughey speculates that the group’s future studies could have important applications for assisted

Collaborative Science

Bertram Jacobs knew what he wanted to do as soon as he saw the advertisement from the National Institutes of Health. The 1991 announcement proclaimed that the NIH would fund research related to the transmission of HIV from mother to child. An ASU professor of microbiology, Jacobs is an expert on interferon, a drug being tested as a treatment for AIDS patients. He wanted to find out what effect interferon might have on both a developing human fetus and on the placenta, the organ that protects and feeds a growing baby inside the womb.

There was one major problem: Jacobs’ ASU lab is located in Arizona, where experiments on human embryonic tissue are illegal. The solution was one common to science: improvise.

Jacobs’ first set of experiments was relatively simple to design. Mouse embryos had long been used as models for human development. But finding something to mimic the human placenta would be more difficult.

“Other than primates, interactions between the mother and the human placenta are very different than the interactions that take place between other animals and their placentas,” Jacobs explains. “Primates are in very short supply and very expensive to use for these studies. As a result, we were left trying to find another experimental model of how the human placenta works.”

The placenta grows from tissue that comes partly from the mother and partly from the fetus. Jacobs could not use aborted or miscarried placenta without risking a lawsuit. He turned to fellow ASU professors and scientists Robert McGaughey and David Capco for help.

McGaughey had done extensive research with physicians working at Good Samaritan Medical Center’s in vitro fertilization clinic. The ASU scientists were able to get placental tissue from women who had just given birth. Using the expertise developed in Capco’s laboratory, they used those samples to grow larger amounts of placental tissue in lab dishes. Then they studied the effects of interferon on that newly grown tissue.

Getting cells to grow outside the body is not an easy task. Jacobs and his colleagues had several problems coaxing the tissue to grow into a continuous layer.

They persevered. Once successful, they found that interferon could halt the reproduction of several non-HIV viruses within the placental tissue. If the findings are extended to HIV, interferon might one day be used as a treatment to prevent HIV from being passed from mother to child.

Collaborative science can be a difficult process. But in this case, the cooperation between scientists from different disciplines paid off. Jacobs says that it would not have happened had the three researchers not met through ASU’s special program for molecular and cellular biology.

“I come from a biochemistry background. Biochemists like to cut things up and separate them and then ask how each piece works,” Jacobs explains. “That's necessary and that's good, but there comes a time when you have to ask how the whole thing works. That's when collaboration becomes important.” — Alana Mikelsen
reproductive technologies such as in vitro fertilization.

“The culture of embryo cells outside the body notoriously results in lower embryo development than usually is seen inside the animal,” McGaughey says. McGaughey collaborates with physicians working in the Piper Clinic at Scottsdale Memorial Hospital North.

“We never know why that is,” he continues. “We always attribute it to general culture conditions being inadequate compared to the conditions found within the female reproductive tract.”

The improved development of the team’s mouse embryos in the presence of interferon strongly implies that adding interferon to embryo cultures might improve the success of in vitro procedures, McGaughey says.

He thinks that interferon in the womb could have an antiviral, protective role similar to its function elsewhere in the body. On the other hand, interferon could be a more fundamental player in cell development itself. The ASU group plans to test mouse embryos for classical signs of interferon regulation, including the presence of receptor molecules that would indicate the embryos could directly receive an interferon signal.

They also plan to see if embryos implanted into a mouse after interferon treatment will develop further than the blastocyst stage at which the previous experiments stopped.

“If we get a baby mouse, then we can be pretty confident that the treatment is minimally damaging,” McGaughey says.

In the meantime, both Jacobs and McGaughey caution that their results are preliminary, and that much more work needs to be done before interferon could be developed into a possible combination therapy for HIV positive women looking to protect their babies.

**STILL NO MAGIC BULLET IN SIGHT** Scientists have only just begun to delve into interferon’s possible effect in the womb, and they have no idea how it works to overcome AZT damage to embryos.

“Embryogenesis—the stage (of development) we’re looking at—is a highly regulated process, and most of the regulation we don’t understand,” McGaughey explains. “If we start playing with embryos and interferons we may unbalance an embryonic mechanism that could cause more damage than good. So I’m talking about years and years of research.”

Facing a virtual void in long-term medical research, society is beginning to turn to legal remedies to prevent the spread of disease to victims commonly considered ultimately innocent. Legislation that criminalizes knowing transmission of HIV through body fluids and sexual contact has been on the books for a number of years. Such laws now are being subtly molded to protect the unborn.

Involuntary screening of pregnant women is the norm in many states, and newborns are routinely tested for HIV without parental consent in nearly all jurisdictions. And although no AIDS legislation specifically criminalizes the prenatal transfer of the disease, anti-transmission laws in Illinois, Arkansas, Idaho and Indiana are worded vaguely enough to make childbearing a felony for an HIV positive woman—even if the fetus is one of the roughly 70 percent who don’t test positive 18 months after birth.

As the public continues to clamor for scientific solutions, Jacobs warns that a cure for AIDS is not likely soon, if ever.

“We are just past the 100th anniversary of the discovery of viruses. To date, we have just one good antiviral drug,” he says, referring to acyclovir, which works against the herpes virus. “I don’t think that’s a coincidence. Finding antiviral drugs is a very, very, very difficult task.

“We’ve wiped one virus off the face of the Earth with a vaccine. That would be smallpox,” Jacobs continues. “If we want to, we can control measles. But we are less good at controlling influenza—and most people compare the potential effectiveness of any possible vaccine for HIV to an influenza vaccine. To think that we’re going to come up with a vaccine for HIV when we’ve not come up with a magic bullet for the flu, or the common cold, or any other virus makes me skeptical,” he says.

“Oh the optimistic side, the reality is that we know how to stop this epidemic. We can stop it now,” Jacobs adds.

McGaughey agrees. The “obvious solution” to the problem of HIV spread is the same today as it was 10 years ago: using condoms during intercourse or abstaining from sex, and sterilizing needles for drug injection. But, he laments, one can’t control human nature, which all too often causes people to avoid simple solutions, particularly in the face of issues dealing with sex. So we’ll have to continue to do it the hard way, with science. And science takes time.

Research on developmental biology, interferon, and viral replication at ASU is sponsored by the National Institutes of Health. For more information, contact Bertram Jacobs, Ph.D., Department of Microbiology, 602.965.4684, or Robert McGaughey, Ph.D., Department of Zoology, College of Liberal Arts and Sciences, 602.965.2349.