Pregnancy, Immunity, Schizophrenia, and Autism

by Paul H. Patterson
Recent evidence shows that this brain-immune conversation actually starts during the development of the embryo, where the state of the mother's immune system can alter the growth of cells in the fetal brain. As we shall see, such alterations can lead to an increased risk of schizophrenia or autism in the offspring.

First let's consider schizophrenia, which is a progressive disorder whose initial psychotic symptoms usually appear in early adulthood. (For a gripping rendering of how psychotic episodes might appear to the sufferer, see Russell Crowe in *A Beautiful Mind.* People with schizophrenia can be seemingly quite normal part of the time, and then have very severe problems, which is a huge difficulty for them—people have tended to blame the victim and wonder why the patient doesn't get him- or herself together and behave properly.

In the last decade or two, anatomical and functional differences between schizophrenic and typical brains have begun to emerge. Magnetic resonance imaging (MRI) scans of the brains of identical twins, one with schizophrenia and one without, have shown that in 90 percent of the cases the twin with schizophrenia has enlarged ventricles, which are butterfly-shaped, cerebrospinal-fluid-containing voids in the center of the brain. One explanation for this enlargement is that the gray matter surrounding the ventricles might have shrunk, meaning the brain has fewer or perhaps smaller neurons. Or the neurons might be more densely packed. An alternative hypothesis invokes an infection—encephalitis, for instance, will expand the ventricles. Schizophrenia does not result from a frank infection of the mature brain, but there are other indications, which I'll come back to, that infections might be involved very early on.

MRI shows anatomical details, but functional MRI, which tracks blood flow, shows brain activity. The more blood moving through a particular part of the brain, the more active it presumably is. In these renderings of functional MRI scans of a schizophrenic patient, the head at far left shows, in yellow, that the auditory cortex lit up when a

Can something as innocuous as the flu cause schizophrenia? Can a pregnant mom's sniffles have lifelong consequences for her unborn child? Does the brain's own immune system play a role in autism? The answers to these and related questions are indeed surprising, and may suggest new avenues for treatment or even prevention.

As we learn more about the connections between the brain and the immune system, we find that these seemingly independent networks of cells are, in fact, continually talking to each other. As an adult, the activation of your immune system causes many striking changes in your behavior—increased sleep, loss of appetite, less social interaction—and, of course, headaches. Conversely, stress in your life (as perceived by your brain) can influence immune function—the brain regulates immune organs, such as the spleen, via the autonomic nervous system.

Opposite: A detail from *The Temptations of St. Anthony* by Hieronymus Bosch (d. 1516). The fantastic—in the strictest sense of the word—figures portrayed here are not unlike some hallucinations reported by schizophrenia sufferers. Courtesy of the Museu Nacional de Arte Antiga, Lisbon.
stereophonic sound was played through earphones. The other head shows the brain activity when the patient pushed a button to signal that he or she was “hearing voices.” The hallucinations only appear in the dominant hemisphere, so in this right-handed patient, only the left hemisphere’s auditory cortex lit up. It used to be said that the voices in their heads were imaginary, but since there is activity in the part of the brain that actually processes auditory information, they really exist, in a sense. Schizophrenics are hearing sounds, as far as their brains know, and it would be very interesting to discover what generates this activity spontaneously.

We know that schizophrenia begins in early development. Statistically, children who will later develop psychosis are more prone to disciplinary problems in school, tend to have lower IQs, and are more likely to be beset with emotional and social problems. The differences are too small to be useful for an early diagnosis, but they’re there. There’s also a surprising delay in the development of motor functions—sitting, standing, walking, and so on.

There’s a genetic component to schizophrenia. The most important risk factor for predicting schizophrenia is having a sibling with the disorder. In the general population, the risk for schizophrenia is approximately 1 percent worldwide. If you have a schizophrenic cousin or uncle or aunt, the risk is doubled, which is not very significant. But if you have an identical twin with schizophrenia, the risk is about 50 percent that you will become schizophrenic as well. But it’s not 100 percent, so it’s not a classical, dominant genetic disease like Huntington’s disease, where a single malfunctioning gene gives you the disorder. Rather, people think there are some six to 12 genes involved, each of which contributes a small amount of risk. In the last couple of years, a number of these genes have been identified, including neuregulin, dysbindin, and one called “Disrupted-in-Schizophrenia,” or DISC1. Furthermore, each of these genes is well known from animal studies to be very important in early embryonic brain development.

There is also an environmental risk component. Being born in the winter or spring months, or being born and raised in an urban area both increase risk. This is consistent with an infectious hypothesis—we tend to get sick more often in the winter and spring, and we’re more likely to sample other people’s germs if we live in a crowded area.

Another important environmental risk factor is maternal infection, which will be one of my major themes. Having a respiratory infection during the second trimester of pregnancy increases the risk for schizophrenia in one’s offspring. In the year 2000, Alan Brown and his colleagues at Columbia University in New York studied the medical records of 12,000 pregnant women who belonged to the Kaiser HMO in the Oakland area. Brown found...
that there was about a threefold increase in risk if the woman had a respiratory infection during the second trimester, confirming the conclusions of previous studies that had not had access to patient records. The researchers then analyzed frozen serum samples from those women, and found a similar, or even larger—up to sevenfold—increased risk if antiflu antibodies were present during the first half of pregnancy. Moreover, they found a statistically significant association with elevated levels of some members of a group of proteins called cytokines. Cytokines are produced by the white blood cells, and their levels in the blood increase when we get an infection. A calculation of the so-called attributable risk from this data led to the estimate that about 20 percent of the schizophrenia cases would not have occurred if flu exposure had been prevented.

This is a really dramatic piece of information, particularly given that the researchers had to completely ignore the genetic angle. (Even now, we cannot screen for the susceptibility genes that have since been identified.) Thus, the study presumably included a large number of people who will never get schizophrenia because they aren’t genetically predisposed, yet it still found a three-to-sevenfold risk increase. The actual risk due to maternal infection is therefore likely to be much higher.

Other studies of adult schizophrenic subjects have found cytokine imbalances and elevated levels of white cells in the blood. And antipsychotic drugs such as clozapine, which people take to treat hallucinations and disordered thoughts, are known from animal studies to modulate cytokine levels in the blood. So these drugs might not only be acting in the brain, but on some aspect of the immune system to achieve their effectiveness. I think this is a very interesting observation, but it hasn’t made much of an impression on the research community yet, so the possibility hasn’t really been investigated carefully.

A recent, very impressive paper by William Eaton and colleagues at Johns Hopkins University Medical School analyzed the remarkably comprehensive records of Denmark’s health system, which tracks every Dane from the cradle to the grave. The investigators accessed the files on all 7,704 people who were diagnosed with schizophrenia between 1981 and 1998, including the details of every hospital visit those people ever made in their entire lives. It turns out that people who developed any of nine different autoimmune disorders—diseases in which the body’s immune system begins attacking one’s own cells—had a 45-percent increase in risk for developing schizophrenia.

So there is a link between the immune system and schizophrenia, but we don’t know what it is. We know that a genetic predisposition to autoimmune disease exists—are the genes responsible for this predisposition somehow linked to the ones predisposing to schizophrenia? Or is there something about having an autoimmune disorder, such as the creation of antibodies against certain molecules, which increases risk for schizophrenia?

Now let’s turn to autism, which was originally described by Leo Kanner at Johns Hopkins in 1943 as a type of schizophrenia. We don’t think that way anymore, but there are some interesting similarities—particularly in the withdrawal of patients from the world around them. The hallmarks of autism are, of course, deficient social skills—patients don’t read other people’s emotions well or respond to them appropriately—and the lack of development of language. Heartbreakingly, about 30 percent of patients actually experience a regression in these areas that starts at about age three. Unlike schizophrenics, however, autistic children frequently display odd, repetitive gestures—banging their heads against the wall, or a flapping motion with the hands that is a classic symptom often used by teachers as a possible indication that a problem may exist. And autistics tend to fixate on objects and rituals. A patient might spend hours playing with a piece of string, for example, or eating her dinner in just the right way. There’s also fear of new situations or objects, and oftentimes considerable problems with sensory stimuli—extreme sensitivity to noises, for example. Alarmingly, cases of autism appear to be dramatically on the rise. However, it’s not clear how much of this actually represents an increase in the incidence of autism, or an increase in the diagnosis of autism rather than, for instance, mental retardation.

Like schizophrenia, there’s a strong genetic component to autism—the single biggest risk factor is having a sibling with it. Autism is also a multi-
genetic disorder, with six to 10 genes involved, and again, the genes that have been identified thus far (neuroligins 3 and 4, En-2, and Hox-a1) are very important in embryonic brain development. Furthermore, there are environmental risk factors for autism. Valproic acid, which is used to treat epilepsy, causes a dramatic increase in the risk of autism when taken by women before they know they’re pregnant. This drug is still commonly prescribed, but people are beginning to get concerned about its use by pregnant women.

We have a valuable insight into the fetus’s period of vulnerability, thanks to the thalidomide tragedy. Those of you who are old enough will remember the use of thalidomide as an anti-morning-sickness drug in the 1960s. Severe birth defects resulted, as did an increased incidence of autism. Valproic acid, which is used to treat epilepsy, causes a dramatic increase in the risk of autism when taken by women before they know they’re pregnant. This drug is still commonly prescribed, but people are beginning to get concerned about its use by pregnant women.

We have a valuable insight into the fetus’s period of vulnerability, thanks to the thalidomide tragedy. Those of you who are old enough will remember the use of thalidomide as an anti-morning-sickness drug in the 1960s. Severe birth defects resulted, as did an increased incidence of autism. But what is key here is that the kind of physical abnormality one got—missing ears, stunted arms, stunted legs—was found to depend on how far along the pregnancy was. In other words, the child’s deformity told us exactly when, sometimes to within a day or two, the mother took the drug. The window of risk for autism proved to be days 20 through 23 after conception—a very early stage in neural development. At this time, the neural tube is just closing, and the first neurons are just being born. A similar window of risk is found with valproic acid, and with an ulcer-preventing drug called misoprostol. We don’t know the cause or causes of autism in most cases, but this window of vulnerability is clearly a very important clue to how the brain is altered in this disorder.

Again, as in schizophrenia, there’s a maternal-infection risk factor. A review of the literature by Andrea and Roland Ciardello at Stanford concluded that “the principal nongenetic cause of autism is prenatal viral infection.” This was based primarily on studies of an epidemic of rubella, or German measles, in New England in 1964. On the order of 10 percent of the children born to infected mothers exhibited symptoms of autism, which is really an astronomical increase in risk. Of course, rubella is not common anymore, because we get vaccinated against it, but the point is that maternal infection can increase the risk for autism. Other infections have also been implicated—a paper just came out last week linking genital herpes infection with an increased risk for autism.

Without going into the details, the rates of autoimmune diseases and allergies are higher in families with autism, particularly in the mother. There are also reports of immune dysfunctions in the blood of autistic individuals. These various connections to the immune system are, of course, reminiscent of schizophrenia.

There is also very striking evidence of immune dysregulation in the brain itself. Just last year, a group led by Carlos Pardo at Johns Hopkins found what they’re calling a “neural inflammation” in postmortem examination of brains of patients with autism who died between the ages of eight and 44 years. But these people weren’t infected—they died of such things as drowning or heart attacks. The study found that the microglial cells, which act as the brain’s own immune system, were activated. The study also found amazing increases of certain cytokines in the brain, and of others in the cerebrospinal fluid. This is a landmark paper, in my opinion. It presents the first evidence that there’s an ongoing, permanent immune-system activation in the brains of autistic people. It’s a subclinical state, because there’s no overt infection. But it’s there.

To try to untangle how the immune system is intertwined with the development of these diseases, we turned to an animal model. Animals are vital to
might be some change in the electrical properties of the neurons, or some molecular change such as the cytokine levels. Or it could be the tremors and shuffling gait of Parkinson's disease. In fact, the mouse models of Alzheimer's and Huntington's diseases that are in routine use in labs around the world do not display some of the diseases' key features. The neurons that typically die in human patients somehow survive, for example. So a model doesn't have to be perfect to be extremely useful, even when testing potential human therapies.

Our laboratory is exploring a model of maternal infection. We give a pregnant mouse the flu by touching a pipette containing a solution of the human influenza virus to her nose, which she then inhales. The mouse gets lethargic, stops grooming herself, hunches in the corner of the cage, and in a few days recovers and behaves normally again. In due time she gives birth, and we study the pups, both as infants and adults. We watch their behavior, and then examine their pathology—what their brains look like.

Fortunately, that isn't what we really do with animal models. We don't mimic the whole disease in any model—we mimic features of the disease. This might be the kinds of neurons that die. It
The path followed by a control mouse exploring an unfamiliar place. The asterisk marks where the mouse was placed in the box, and the red circles show where the mouse stood up on its hind legs for a better whiff of its surroundings.

By contrast, a mouse whose mother got the flu tends to stay hunkered down in the corner where it was placed.

lines on the floor so we can track where the animal moves. A normal mouse usually spends a lot of time creeping along the edges of the box at first, because it’s afraid that it’s dangerous to go out into the middle—which, obviously, it might be. But it will eventually inspect most of the box, pausing frequently in the process to rear up on its hind legs and sniff the air. Our normal mice, which we call control mice, are born to “sham-infected” mothers who were given a sterile saline solution without the virus. These mice do exactly the same thing—they are timid at first, but they’re soon traipsing all over the box.

Below is an example of an adult mouse who was born to a flu-infected mother, and you can tell immediately from the fecal pellets that it hasn’t moved beyond its corner very much at all. We would interpret this as excessively fearful behavior, given the mildly stressful nature of the situation, and we can quantitate it by simply measuring the amount of time spent in the center squares of the box. This mouse enters the center many fewer times, and it rears and sniffs much less often as well.

The so-called novel-object test is also relevant. Remember, autistic children are often afraid of unfamiliar things. So when we put something strange and new in the field, say a coffee cup, the control mouse carefully investigates it, touching and sniffing it from all sides, whereas our mouse born to an infected mother is very reluctant to go anywhere near it. In fact, this mouse turns its head away and acts as if the object isn’t there. We measure the time lapse before the mouse first touches the object, which we call the latency to first contact, and we count how often contacts are made. Again, the differences are dramatic. The “autistic” mouse waits much longer, and touches the object far fewer times.

We also do simple social interaction tests. We put two mice who don’t know each other in the box, and ask how long it takes them to make physical contact, and how often they do so. And not surprisingly, pairs of mice born to infected mothers make contact less than half as often and have more than four times the latency. So clearly they’re not socializing properly. Grad student Steve Smith is now following up on that observation by
Top: A normal mouse inspects an unfamiliar object with avid curiosity.

Bottom: An “autistic” mouse ignores it, seeming to act on the theory that if it can’t see the object, the object doesn’t exist.

At least rodents don’t run up bar tabs—biology staff member Limin Shi puts a pair of mice in a three-room box designed to test their social skills.

using a box divided into three rooms. We put our test mouse in the middle room, and then we put an unfamiliar mouse in one of the side rooms. We leave the room on the opposite side empty in some tests, and in others we put a cage mate of our test mouse in there. Then we sit back and watch where our test mouse goes. Normal mice like novelty, and almost always go to the strange mouse, even when there is a familiar mouse in the other room. Preliminary results with our “autistic” mice, however, show that they prefer to remain in the central chamber regardless of who else is in the box with them.

Another pertinent test is the startle response, which is a lot like sneaking up behind someone with an inflated paper bag and popping it. We put the mouse in a tube inside a soundproof box, and underneath that tube is a motion sensor. There’s a speaker in the box, and when a loud sound is played, the mouse is startled, and we measure how high it jumps. But if we precede the loud sound with a softer sound that doesn’t startle the mouse—called a prepulse—it doesn’t jump so much. This is called prepulse inhibition, or PPI, and when the same type of test is done in people, a striking deficit is observed in schizophrenic and autistic subjects. In other words, they get startled just as much regardless of whether they got a prepulse or not. The loud noise surprises them every time. We think this relates to the attention-deficit issues. On the next page is a plot of the amount of the mice’s PPI versus prepulse intensity. As we increase the prepulse intensity, we get a bigger inhibition across the board, but our “autistic” mice have a PPI deficit at every intensity.
The PPI is thought to be a measure of sensory-motor gating—the connection between the filtering of incoming sensory information and the creation of motor outputs to the muscles—which is likely to be related to attention deficits and distractibility. In fact, a PPI deficit is also found in attention deficient disorder. Importantly, those antipsychotic drugs mentioned earlier can restore the PPI in schizophrenic subjects, whereas psychomimetic drugs—hallucinatory drugs—disrupt PPI. We have shown the same thing to be true in our mice.

We presume that these behavioral abnormalities are based in brain pathology—changes in the nerve cells, or in their connections. In fact, postmortem examinations of at least some brains of schizophrenia patients have shown nerve cells that are not in their appropriate locations. So recently, biology staff member Limin Shi, postdoc Natalia Malkova, and Steve Smith have been looking at fetal brain development in the mice. For this analysis, the pregnant mice are given the flu at mid-pregnancy, day 9.5 of gestation, which corresponds to the period of very early brain development in humans. In other words, it’s similar to the thalidomide window of autism vulnerability. However, because fetal mice develop so fast, the illness also extends through the period corresponding to that second-trimester stage in humans when maternal infections lead to an increased risk of schizophrenia. Five days into the infection, a dye that marks newly formed neurons is injected into the mice, and they give birth six days after that. At right is the brain of a normal pup. The green neurons have taken up the dye, and most of them have migrated out to what neuroanatomists call layers 2 and 3 of the cerebral cortex. This is similar to how a normal newborn human brain would look, too. But this layer is barely present in the pups from infected mothers. Something has gone very wrong, because the green cells have wandered off all over instead of forming the normal, tightly packed layers. We plan to repeat the experiment but let the pups grow to adulthood to see if this disorganization persists, and whether it looks similar to what was found in those few human schizophrenia examples.

Another human pathology occurs in the cerebellum. The cerebellum has lobes, called lobules, which look like a cauliflower in cross section, and contain neurons called Purkinje cells that are pres-
Neurons from an early stage of brain development have been labeled with a fluorescent green dye. These neurons form clearly visible layers in a healthy newborn mouse brain (top), but when the mother was infected in midpregnancy (bottom), the neurons are scattered almost at random.

There is a fascinating correlation between abnormalities in lobules VI and VII and children's exploratory behavior. In 2001, Karen Pierce and Eric Courchesne at UC San Diego did a study where they put a child (aged three to eight) in a room with a lot of brightly colored boxes and other intriguing objects, and counted how many of them the child played with in eight minutes. The control kids, on average, explored about 10 of the 14 items. But the autistic children tended to fixate on a few objects to the exclusion of all others—in one extreme example, the child got no further than the very first item it encountered. All of these children had previously had MRI scans as part of another study, and a dramatic correlation popped out—the smaller the autistic child's lobules VI and VII, the fewer objects the child showed interest in.

Because our “autistic” mice were similarly immune to the allure of an unknown object, we wanted to see if they had the same cerebellar abnormality. Treating the cerebellum with a dye that just stains Purkinje cells reveals a consistent difference in these mice, as you will see on the next page. In addition, we occasionally see what we think are misplaced Purkinje cells. The cell bodies are supposed to line up in a neat row along the boundary between the red and the black zones, and not dawdle in the dark interior. We think that this misplacement must have occurred in embryonic development.

Now let’s consider the mechanism for how this works, which is where the animal model comes in very handy indeed. Does the virus actually infect the fetal brain itself, or is it working indirectly through the mother’s immune system? We think...
it’s the latter, because we can’t find the virus in the offspring, either in the embryonic brain or at birth. That’s not surprising because, after all, influenza is primarily a respiratory virus. It hardly ever gets out of the lungs, throat, and nose and into the rest of the body. When it does, you have viremia, which is a very serious disease.

Furthermore, we can evoke an immune response in the mother without using a virus, simply by injecting her with a piece of double-stranded RNA. Mammals don’t make double-stranded RNA but many viruses do, so the immune system knows that when it sees double-stranded RNA, it needs to swing into action. It starts secreting cytokines and in general mounting a vigorous antiviral response, even though there’s no infection. Tellingly, the offspring of mothers whose immune systems have been artificially activated in this way display the same PPI deficit that we saw before. So we don’t need the virus; activation of the maternal immune system is sufficient to alter the behavior of the offspring.

A second example of “autistic” behavior brought on by maternal immune activation was discovered by Natalia Malkova. Anecdotal evidence suggests that autistic human infants may be less bonded with their mothers. When Natalia removes the mother mouse from the family cage, it normally induces considerable crying in the control pups, although since mouse pups vocalize at ultrasonic frequencies, we have to use a special microphone to hear them. So Natalia counted how often the pups cried in three minutes, and the mice born to a double-stranded-RNA-exposed mother cried less than pups born to a normal mother.

We think that maternal immune activation alters brain circuits. Besides that dramatic abnormal layering Limin finds in the mouse cortex, and a loss of Purkinje cells that’s been seen in the human cerebellum, there’s that permanent, subclinical, altered immune state in the autistic brain—those increased cytokine levels. Are those cytokines an irrelevant, residual footprint—a fossil, if you will—of some earlier event, like a maternal infection? Or are they actually interacting with the brain in an ongoing fashion, with consequences visible in the patients’ behavior? I favor the latter hypothesis.
In some clinical trials where cancer patients were given cytokines in the hopes that these molecules would attack their tumors, dramatic differences in behavior and mood became apparent—up to severe depression, in the worst cases. And other researchers have found that high levels of cytokines in animals can alter learning and memory.

If this hypothesis is true, what would happen if we changed the brain’s immune state? Antipsychotic drugs are known to suppress the immune system. Is that relevant to psychotic behavior? We are very interested in this possibility. In fact, Carlos Pardo of Johns Hopkins and I are organizing a meeting with the Cure Autism Now and Autism Speaks foundations to examine the possibility of immune intervention in autism. People take anti-inflammatory drugs such as aspirin to modulate their immune response all the time—is this a strategy worth exploring in this context?

We are just starting to explore the interactions between the immune system and the developing brain. Cytokines aren't the only possible conduit from a mother's infection to the fetus's developing brain—there are other changes brought about by corticosteroids, which are released following an infection or sickness, that also have effects on the fetus. And don't forget the genetic component—on what are those genes acting to increase the susceptibility? They might affect fetal brain development directly, or they might affect the brain's susceptibility to such other factors as cytokines, or the response of the placenta to the mother's immune activation, or they might even be acting in the mother, to affect her response to infection. We should be able to sort these possibilities out eventually, using this animal model.

Finally, I want to ask a question that’s come up in the literature in the last few years—should we really be promoting universal maternal vaccination? The flu vaccine has been recommended routinely to pregnant women in the United States since 1957. The official policy of the Centers for Disease Control states that “administration of vaccines to women seeking prenatal care is an opportunity for preventative intervention that should not be wasted.” Now you might say, “Well, of course, you don't want to get the flu if you're pregnant!” But remember that double-stranded RNA experiment—we activated the immune system, and it caused all these downstream effects on the fetus. And what does a vaccination do? It activates the immune system. That’s the point of vaccination. In practice, not all pregnant women receive flu shots, and I think that universal vaccination of pregnant women could get us into a whole new set of problems. I’m hoping, therefore, that a way will be found to intervene somehow and repair the damage or re-regulate the immune system. This mouse model is an excellent place to start.

Top: Purkinje cells (purple-blue dots) in a normal human cerebellum. Bottom: Nine of the 10 autistic brains analyzed showed patchy Purkinje cell loss.

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