Bird Chimeras May Be Models for Certain Neurological Diseases

Quail-chick chimeras with transplanted neuronal tissue mount a prolonged immune response to the graft and reject it, resulting in tissue damage similar to that in multiple sclerosis.

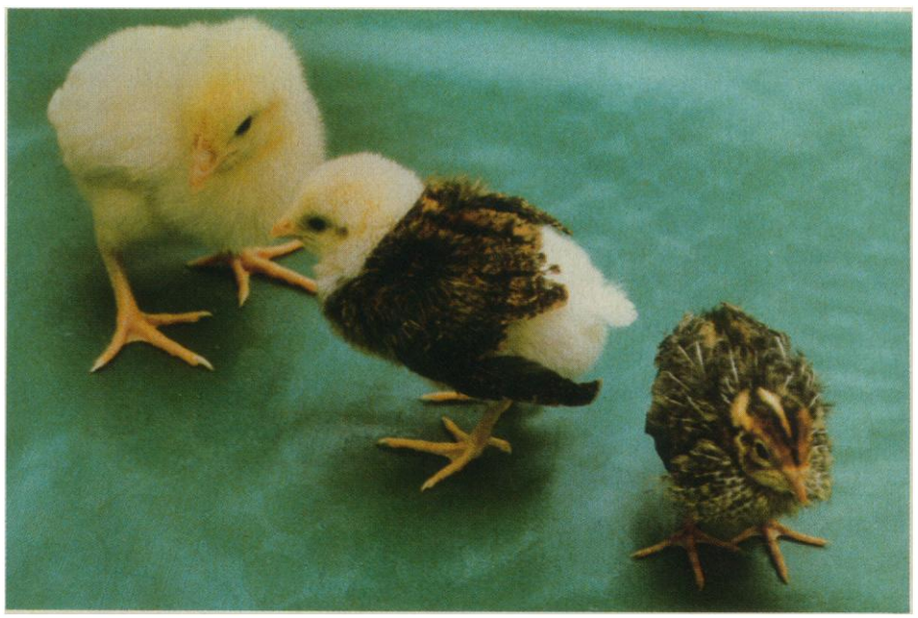
CHICK embryos that receive grafts of embryonic quail nervous system tissue will hatch and look normal for a while. But after a few months, they "develop a neurological syndrome that at the cellular level is very similar to the degenerative changes seen in human multiple sclerosis," says French developmental biologist Nicole Le Douarin. Immune rejection of the grafted tissue leads to paralysis that begins in the birds' wings and soon includes their legs.

In multiple sclerosis (MS), the body seems to mount an immune attack against its own brain and spinal cord tissue. A major consequence of the autoimmune attack is selective destruction of the fatty myelin wrapping around nerve fibers. As a consequence, non-neuronal cells called astrocytes divide in the affected area, forming a scar of sclerotic (hard) tissue. These lesions occur in many places throughout the white matter of the central nervous system, which is why the disease is called multiple sclerosis. There is a loss of nerve function and the disease can be devastating if large areas of central nervous system tissue are affected.

Le Douarin reported her new results at the annual meeting of the American Society for Neurochemistry* and in the 25 April issue of *Cell*. She has dual appointments at the Institut D'Embryologie of the Centre National de la Recherche Scientifique and the Collége de France in Nogent-sur-Marne.

"Patients with multiple sclerosis have many different symptoms, depending on the location of their lesions. These may be in the brain, brain stem, spinal cord or optic nerves," says Ute Traugott of the Albert Einstein School of Medicine. "As a result patients may be clumsy, have an unsteady walk, may be unable to walk, have problems with urination, and have transient blindness or double vision." To diagnose a patient as having MS, doctors look for at least two of the above symptoms, indicating the presence of multiple lesions.

The cause of MS is unknown, says Robert Fallis of Brigham and Women's Hospital and Harvard Medical School in Boston. "A guess is that a virus may induce an initial immune response in the brain that isn't shut down properly in genetically predisposed individuals. But the problem with this notion is that a common viral antigen has never been found."



Chick (left), quail-chick chimera (center), and quail (right) at similar ages. The pigmented feathers are due to a graft of embryonic quail nerve tissue.

One animal model that has provided insights into the pathologic mechanisms in MS is experimental allergic encephalomyelitis (EAE), which involves an inflammation of the brain and spinal cord. To induce EAE, researchers inject white matter, myelin basic protein (a protein component of myelin), or even T lymphocytes from a sick animal into a test animal. Depending on what is injected, there are pathological changes in the test animal's central nervous system that resemble different forms of human MS.

Le Douarin and her colleagues, Monique Coltey, also at the Collége de France, and Masae Kinutani, now at Ehime University School of Medicine in Shigenbou, Japan, use a different approach in their new bird model for MS. Instead of injecting an adult animal with myelin-containing tissue or lymphocytes, they transfer pieces of neural primordium from a Japanese quail embryo into an embryo of another species, the White Leghorn chicken.

The neural primordium is a long tubular structure along the embryo's back that differentiates into the brain and spinal cord (central nervous system). It also includes neural crest cells, which appear at the sides of the neural tube very early in embryonic development. In her previous work with the quail-chick chimera system, Le Douarin showed that neural crest cells migrate and differentiate to form many neuronal and nonneuronal tissues, including the entire peripheral nervous system. Grafted neural primordium supplies the chick with two kinds of nervous system tissues—central and peripheral.

This is important because peripheral nervous system tissue, unlike the spinal cord and brain, is not protected from immune system cells by the blood-brain barrier. Le Douarin proposes that chicks with transplanted quail neural tissue reject it because

*The annual meeting of the American Society for Neurochemistry was held in Montreal, Quebec, 16 to 21 March 1986.

"the immune attack starts in the peripheral nervous system where there is no blood-brain barrier to protect the tissue. Normally, cells of the immune system, including T lymphocytes [which mediate graft rejection] are circulating in the blood and are not in contact with the cells of the central nervous system." She thinks that after the chick begins to reject its peripheral nervous system tissue the disease spreads to grafted spinal cord, probably because of damage to the blood-brain barrier and the ability of T lymphocytes to enter the central nervous system.

Le Douarin became involved in her recent work on the immunological status of the transplanted tissue partly because of comments made by scientists who heard her present information about neural crest cell differentiation. Whenever she spoke about the development of the peripheral nervous system and other tissues from neural crest cells, someone in the audience would ask about the fate of grafted tissue in young or adult chimeras. This question led her to the present experiments and her discovery that spinal cord chimeras may be a model for MS.

In previous work with neural crest cell differentiation and in new work with the development of the immune response in quail-chick chimeras, the French group capitalizes on the different appearance of quail and chick cells. When properly stained and examined under a microscope, quail cell nuclei contain a dense and compact mass of heterochromatin and are easily distinguishable from the larger, lighter staining chick cell nuclei.

Throughout embryogenesis the chicks tolerate their quail neural primordium grafts completely and eventually hatch. "The only sign that you have a chimera is a stripe of quail-like pigmented feathers," says Le Douarin. "The chickens are healthy at first; they can stand, walk, and fly. Also, the spinal cord and peripheral nervous system arising from the graft are perfectly well developed."

"But," says Le Douarin,"at times that vary from 1 to 4 months, the chickens develop neurological problems." First, their wings droop and become paralyzed. "A few days later, they have difficulty standing and end up with a spastic paralysis of their legs." When the team looks at the region of spinal cord derived from the transplant at this stage of rejection, they see abnormalities that signal immune rejection.

The grafted spinal cord shows changes similar to those seen in the active plaques of MS, including fluid accumulation and tissue swelling, rupture of the blood-brain barrier, and an invasion of the grafted neural tissue by B and T lymphocytes and macrophages. These inflammatory cells typically surround blood vessels and have chicken class II major histocompatibility antigens on their surfaces, indicating that they come from the host and not the graft. Also, areas of the fatty myelin sheath that normally surround nerve fibers degenerate, a demyelinating condition that interrupts nerve function.

"I interpret these findings as a rejection of the graft by the host. But the rejection begins at several months of age, long after the host is immunologically competent," Le Douarin reports. She thinks that the chick's blood-brain barrier protects grafted quail spinal cord for at least several weeks, making it "immunologically privileged," during this time. But, as the blood-brain barrier breaks down, immune cells invade and destroy central nervous system tissues arising from the transplant.

In the most severely affected chickens, the disease spreads beyond the graft and includes the host's own spinal cord tissue. At this stage, the animals appear to have an autoimmune disease in which T lymphocytes and macrophages attack many regions of the spinal cord. A similar immune attack occurs in MS patients and in animals with certain forms of experimental allergic encephalomyelitis.

But there are differences between Le Douarin's model and human MS. For example, "in the human disease," says Traugott, "the symptoms can appear and then go away, sometimes for long periods. This remission period is a mystery. Or, in another form of the disease known as chronic progressive multiple sclerosis, the symptoms get progressively worse." Le Douarin's spinal cord chimeras do not exhibit periods of remission, perhaps making them a better model for the chronic progressive phase of MS.

A second difference involves the regions of the central nervous system affected by the disease. Le Douarin thinks that, in bird chimeras, immune rejection begins against peripheral nervous system tissue and then spreads to the spinal cord. But "peripheral nervous tissue is usually spared in multiple sclerosis patients," according to Traugott. "The peripheral nervous system autoimmune disease is called Guillain-Barré syndrome, and it usually does not overlap with MS."

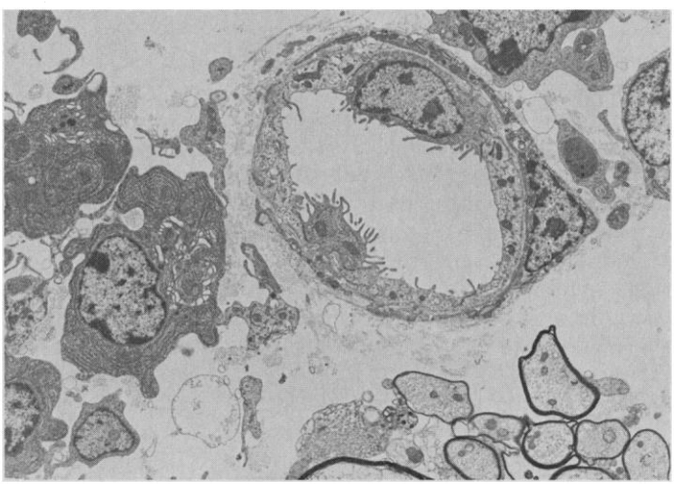
A third distinction between human MS and the demyelination disorder in spinal cord chimeras is that, in the human disease, lesions occur in the brain and spinal cord, whereas lesions in bird chimeras are initially confined to the grafted tissue. Only in its later stages does the disease in chimeras spread to areas of the spinal cord outside the graft.

But no animal model for human MS is perfect, including the most widely accepted model, experimental allergic encephalomyelitis. "The early versions of this model were acute ones," says Henry McFarland of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS). "You would take white matter from an animal, grind it up, and mix it with something that enhances the immune response. When this material was prepared from one guinea pig and injected into an-

Quail-chick chimera at 37 days. Its wings are paralyzed and droop because the bird is rejecting its spinal cord graft.

other identical guinea pig, the second animal became paralyzed within 10 days and later died. These animals had large numbers of lymphocytes that had migrated into the nervous system."

Today, the techniques for inducing EAE are more sophisticated. Dale McFarlin, also at NINCDS, and Cedric Raine, at Albert Einstein College of Medicine, collaborated to develop a recent model for MS in mice. Damage to the central nervous system in these mice becomes permanent, and the disease becomes progressive by expanding into larger and larger regions of the spinal cord and brain as it does in MS.



Electron micrograph of grafted quail spinal cord from a chimera that had both wing and leg paralysis. Plasmocytes (left and top right) are around the blood vessel (top center), and several nerve fibers (lower right) have less myelin than normal. ×3670

Fallis works with a mouse model very similar to one developed by McFarlin. He takes lymph node cells from a mouse that already has experimental allergic encephalomyelitis and injects the lymphocytes and macrophages into a second mouse. The second mouse develops a form of experimental allergic encephalomyelitis that resembles the early stage of MS in which symptoms come and go. To transfer EAE from the first mouse to the second, a subset of helper T cells must be present in the lymph node preparation. "If this subset of T cells is removed, the naïve mouse doesn't get sick," says Fallis.

Fallis and his colleagues follow the course of EAE in the second mouse by looking for lymphocytes that react to the original antigen, myelin basic protein. "These reactive cells appear to be T cells and are in the periphery, even though experimental allergic encephalomyelitis is a central nervous system disease. EAE may be a systemic disease that is manifested in the central nervous system," says Fallis.

There are major unanswered questions concerning MS. T lymphocytes are present in the brains and spinal cords of MS patients, but how do they get there? "Some people think that T cells have the capacity to break down the blood-brain barrier. Others think that T cells squeeze through it, perhaps because of a local inflammation at the level of the barrier," says Fallis.

And what stimulates T cells to attack central nervous system myelin after they get through the blood-brain barrier? In order to mount the abnormal immune response, T lymphocytes must simultaneously "see" both brain antigens and antigens made by the major histocompatibility complex (MHC) genes. This means that some cell type in the central nervous system has to present both kinds of antigens to the T cells. "There are two candidates for this, endothelial cells that line brain capillaries and astrocytes," says McFarland.

Traugott has demonstrated that, in a mouse EAE model and in MS tissue, astrocytes and endothelial cells have a common ability to express class II antigens coded for by MHC genes and to present brain antigens to T lymphocytes. After T cells are stimulated in this manner, they secrete factors that signal macrophages to invade brain tissue, and demyelination results.

What McFarland finds intriguing about Le Douarin's bird chimera model for MS is that "it may provide a suggestion about the mechanism for the disease. That is, you may not need myelin or its basic protein as an antigen." It may be enough to trigger T cells and macrophages to migrate into central nervous system tissue and cause demyelination. ■ **DEBORAH M. BARNES**

ADDITIONAL READING

M. Kinutani, M. Coltey, N. M. LeDouarin, "Postnatal development of a demyelinating disease in avian spinal cord chimaeras," *Cell* **45**, 307 (1986).

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U. Traugott, E. L. Reinherz, C. S. Raine, "Multiple sclerosis: Distribution of T cell subsets within active chronic lesions," *ibid.* **219**, 308 (1983).

Briefing:

Cancer Progress Data Challenged

For several years, statistician John Bailar of the Harvard School of Public Health has been a thorn in the side of the National Cancer Institute. The NCI has said its goal is to reduce age-adjusted cancer mortality by 50% by the year 2000. That goal is "unlikely," Bailar says because "overall cancer mortality is going up." The problem, according to Bailar, is not that cancer treatments are ineffective but that they are not getting better. Now Bailar and his colleague Elaine M. Smith of the University of Iowa Medical Center in Iowa City make their argument in the 8 May issue of the *New England Journal of Medicine*.

Bailar and Smith's data are not in dispute. They come from the National Center for Health Statistics and they indicate that, from 1962 to 1982, age-adjusted cancer mortality rates in the United States increased from 170.2 to 185.1 per 100,000. Yet no one denies that there has been remarkable progress in treating some cancers. Childhood cancers and Hodgkins disease, for example, are remarkable success stories. But these successes are almost washed out in the statistics because they represent such a small proportion of cancers. According to Vincent DeVita, director of the NCI, "50% of all cures through chemotherapy occur in 10% of all cancer patients." Most cancer deaths are from just a few kinds of cancer, particularly lung cancer, which dominates the bleak cancer statistics. Early detection programs for lung cancer have not been successful and the cancer continues to have a grim prognosis.

Yet even if the lung cancer data are removed from the cancer mortality rates, there is no dramatic difference in the overall picture, Bailar and Smith argue. They deleted the lung data and report that the age-adjusted cancer mortality rate since 1950 shifts from an 8% increase to a 13% decrease. But it is only fair, they suggest, to also delete cancers of the stomach and cervix from the data because these cancer survival rates also changed, but for the better, for reasons having nothing to do with treatment advances. (Stomach cancer incidence has been declining for unknown reasons and cervical cancer incidence has declined largely because, it is suspected, of widespread Pap smear screening.) If these two cancers are also excluded from the total picture, the cancer mortality rates stay essentially the same from 1950 until 1982.

Statisticians caution, however, that care