itself] that tend to confirm the solar cycle hypothesis."

Almost by default, the expedition members came down on the side of a chemical explanation for the formation of the hole. Catalytic destruction by man-made compounds has been a leading contender if only because the hole has been deepening in proportion to the increase of chlorine-containing compounds in the atmosphere. Those chemical analyses made by the expedition that were complete or in preliminary form by mid-October were at best consistent with catalysis of ozone destruction by chlorine, but no particular chemical theory could be proved or disproved. "We believe that a chemical mechanism is fundamentally responsible for the formation of the hole," said expedition leader Susan Solomon of the National Oceanic and Atmospheric Administration's Aeronomy Laboratory in Boulder, "but what's happening is more complicated than what has been proposed so far." One complication may be chemical reactions on stratospheric cloud particles, the products of the reactions not being released until the particles evaporate when sunlight hits them in the spring.

Things will certainly get more complicated before the mystery is solved, as suggested by the size of the November special issue of *Geophysical Research Letters*. It contains 46 papers that bring together a variety of observations and theories bearing on the hole. Already some of the papers are being cited in support of one view or another. Schoeberl notes that several papers report evidence of a small climate change in the stratosphere since 1979. And ozone decreases seem to be correlated with temperature decreases. Thus, the temperature change might have led to the progressive strengthening of winds into the polar stratosphere and the intensification of the hole, says Schoeberl. In that case, a dynamic mechanism could both create a hole each October and intensify it from year to year.

Everyone agrees that the atmospheric circulation over Antarctica makes the stratosphere there special. Atmospheric circulation may be solely responsible for the hole and its intensification, in which case the hole could become merely a scientific curiosity. Or the circulation may be creating special conditions under which particularly voracious chemical reactions occur. If those reactions can occur elsewhere in the future when chlorine concentrations are still higher, the entire global ozone layer could be in greater danger than previously thought. With such a crucial distinction to be made, researchers will probably be taking at least the next 2 or 3 years to come to a decision. ■

RICHARD A. KERR

Drug Resistance of Cancer Cells Probed

A better understanding of cancer cells' defenses against chemotherapeutic drugs is beginning to point the way to improved therapies

ALTHOUGH some cancers, especially certain blood cell cancers, can be cured by drug therapy, many of the more common malignancies respond poorly to chemotherapy. For some malignancies, of which colon cancer is a notable example, the drug resistance appears to be an inherent property of the tumor cells. For other cancers, the resistance develops in response to treatment with chemotherapeutic drugs. But however the resistance arises, the all too common result is treatment failure and death for the patient. "Drug resistance is the most important and challenging topic in cancer treatment research today," says Bruce Chabner, who heads the Division of Cancer Treatment at the National Cancer Institute (NCI).

The recent Bristol-Myers Symposium* had as its topic the current progress toward understanding the biochemical causes of drug resistance. "We can now focus on a spectrum of mechanisms—at the membrane level, at the cytoplasmic level with the glutathione system, and at the level of DNA repair and gene amplification," notes symposium co-organizer Paul Woolley of Georgetown University School of Medicine.

Perhaps not surprisingly, the defenses that help cancer cells to survive treatment with chemotherapeutic drugs often reflect the innate ability of cells to protect themselves against damage by foreign chemicals. These defense systems may already be strong in the cells that give rise to inherently resistant cancers. However, drug-susceptible tumors have shown a remarkable ability to adapt to exposure to chemotherapeutic agents by increasing the activity of the defenses.

The past year has seen a great deal of progress toward understanding the origins of multidrug resistance, a common occurrence with current chemotherapeutic regimens. Clinicians frequently find that a patient's tumor will initially shrink in response to treatment with a particular drug or drug combination, but after some period of time will begin growing again. The tumor will then prove to be resistant not just to the drugs with which the patient was treated, but to additional, unrelated drugs as well.

Enhancement of a membrane-level defense produces at least some cases of this type of multidrug resistance. Researchers have known for several years that one of the hallmarks of multidrug resistance is an enhanced ability of the cells to expel or pump out chemotherapeutic drugs. Now several groups have cloned genes for the pump molecule, a membrane glycoprotein, called the P-glycoprotein (where the P stands for "permeability") that is present in higher than normal amounts in the membranes of multidrug-resistant cells.

The groups approached the cloning from different directions. Victor Ling and his colleagues at the Ontario Cancer Institute in Toronto, who originally linked the P-glycoprotein to multidrug resistance in 1976, began with the glycoprotein itself.

Meanwhile, Michael Gottesman and Ira Pastan of NCI, in collaboration with Igor Roninson of the University of Illinois College of Medicine in Chicago and David Housman of the Massachusetts Institute of Technology in Cambridge, had found that cells that display multidrug resistance have increased copy numbers of a gene that they designated *mdr*. Gene amplifications occur frequently in drug-resistant tumor cells and may directly produce the resistance. The classic example of this is resistance to the drug methotrexate, which kills cells by inhibiting an enzyme needed for making the purine building blocks of DNA. Robert Schimke and his colleagues at Stanford University School of Medicine have shown that tumor cells can overcome the inhibition by amplifying the number of genes for the enzyme, which is called dihydrofolate reductase, and making more of the enzyme.

In any event, Gottesman, Pastan, and Roninson obtained circumstantial evidence indicating that the gene encodes the P-glycoprotein. Overproduction of the glycoprotein as a result of the gene amplification might therefore account for the cells' drug resistance. The investigators then went on to

*The Ninth Annual Bristol-Myers Symposium on Cancer Research was organized by the Vincent T. Lombardi Cancer Research Center of Georgetown University School of Medicine and held in Washington, D.C., on 15 and 16 October.

clone and determine the complete nucleotide sequence of a human *mdr* gene.

The supposition that the P-glycoprotein is encoded by the gene has been confirmed, according to data presented at the symposium by Ling and Gottesman. For one, the cloned DNA's of the two groups readily hybridize with one another, which indicates that both represent a P-glycoprotein gene.

For another, Ling's description of the overall structure of the protein product of his group's cloned gene sounds remarkably similar to that presented by Gottesman for the *mdr* gene. "Our molecule looks identical to the molecule that Victor showed," Gottesman says. Moreover, a third group, which includes Housman and Philippe Gros of McGill University in Montreal, has recently cloned and sequenced a mouse *mdr* gene.

The structure of this gene product closely resembles that of the other two. The three genes may not be identical, because there is more than one P-glycoprotein gene per cell, but they are members of the same family.

The results of the three groups show that the protein component of the P-glycoprotein contains nearly 1300 amino acids and has a molecular weight of about 140,000. The molecule can be divided into two nearly equal segments having similar amino acid sequences. This structure suggests that the complete gene arose as the result of a duplication event.

The P-glycoprotein displays a number of structural features characteristic of known transport proteins. Each of its two halves contains a hydrophilic region that probably projects into the cytoplasm and a hydrophobic region that is likely to be embedded in the cell membrane. Moreover, as Ling told the symposium participants, "The transmembrane region goes in and out of the membrane in a way characteristic of pore-forming proteins." The cytoplasmic portions of the molecule resemble certain bacterial transport proteins. The P-glycoprotein can also bind chemotherapeutic drugs.

All of this is consistent with the proposed role of the P-glycoprotein in producing drug resistance, but some of the best evidence comes from Gros, Housman, and their colleagues. By transferring their cloned *mdr* gene, which is apparently a normal P-glycoprotein gene, into drug-sensitive hamster cells, they showed that high expression of the gene is sufficient to confer multidrug resistance on the cells.

June Biedler and her colleagues at Sloan-Kettering Cancer Center in New York City have noted a number of other changes in membrane and cytoplasmic proteins in multidrug-resistant cells, in addition to the increases in the P-glycoprotein. Although these other changes might contribute to the cells' lack of susceptibility to drugs, Biedler says, "The critical change in the cell is the change in the glycoprotein."

The work on the identification and isolation of the P-glycoprotein and its gene was done with tumor cells growing in culture. How extensively increased synthesis of the molecule contributes to the development of drug-resistant tumors in patients remains to be determined. However, it will not account for all cases as other resistance mechanisms can also come into play. Nevertheless, evidence presented by Ling and Gottesman suggests that some human cancers may be drug-resistant because of increased production of the P-glycoprotein.

Another unanswered question about the P-glycoprotein concerns its primary physiological function. Normal, drug-sensitive cells contain the molecule, although in much lower concentrations than drug-resistant cells. The P-glycoprotein's main role might be as a transport molecule for ridding the cell of foreign chemicals. Alternatively, it might move some as yet unidentified endogenous molecule in or out of the cell, in which case the drug transport could be just a fortuitous effect.

One intriguing possibility is that P-glycoprotein is part of the long-sought calcium channel. Certain calcium-channel blocking drugs, which are so called because they prevent the movement of calcium into cells, potentiate the ability of chemotherapeutic drugs to kill resistant cells. The calcium-channel blockers apparently do this by binding to the P-glycoprotein and inhibiting the outward transport of the drugs. However, the reversal of multidrug resistance by the agents may not be a direct effect of their calcium-channel blocking effects. More work will be required to clarify this situation.

The glutathione system for detoxifying drugs in the cytoplasm also received a great deal of attention at the Bristol-Myers Symposium as a possible contributor to drug resistance. Glutathione contains three amino acids; glutamic acid is linked to cysteine, which is linked to glycine. The molecule has a general protective function in the cell.

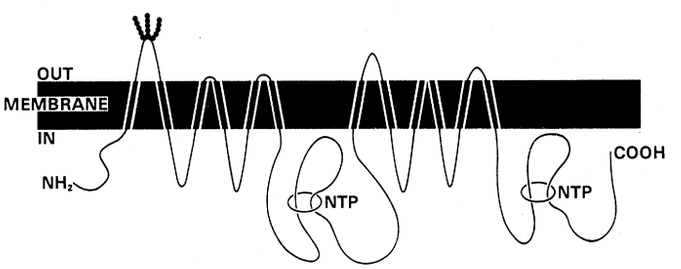
Some chemotherapeutic drugs and radiation generate free radicals and peroxides that contribute to cell killing by damaging the DNA and other essential cell constituents. Glutathione helps protect against this damage by reacting with the free radicals and peroxides. In addition, many foreign chemicals are detoxified by being enzymatically attached to the sulfur on the cysteine of glutathione.

Treatments that decrease glutathione concentrations may potentiate the effects of chemotherapeutic drugs. "Cells with low glutathione concentrations are more sensitive to drugs and radiation," explains Alton Meister of Cornell University Medical College in New York City. He has shown that glutathione concentrations in experimental animals and in cultured cells can be lowered by treatment with the chemical buthionine sulfoximine, which inhibits one of the enzymes needed for glutathione synthesis. Moreover, the agent is relatively nontoxic.

Robert Ozols of NCI is also investigating the possible use of buthionine sulfoximine for potentiating the effects of chemotherapeutic drugs. He has found, for example, that a combination of this agent with melphalan, a drug used for treating ovarian cancer, is superior to melphalan alone in prolonging the survival of nude mice that received transplants of human ovarian cancer cells. Ozols predicts that the combination therapy may be ready for preliminary testing in human patients in 6 to 7 months.

The enzyme glutathione transferase helps to detoxify drugs by conjugating them to the cysteine of glutathione. Increased concentrations of this enzyme may also contribute to drug resistance in tumor cells. For example, Kenneth Tew of the Fox Chase Cancer Center in Philadelphia finds higher amounts of the enzyme in drug-resistant cultured cells than in sensitive cells. When he analyzed biopsy samples taken from human cancers, he found that the amount of the enzyme correlated with the intrinsic drug resistance of the cancers. Head and

Model of the human P-glycoprotein



Most of the glycoprotein, which is divided into two roughly equal parts, is either embedded in the membrane or projects into the cytoplasm. The small exterior portion carries the attached sugars. The "NTP's" indicate binding sites for nucleoside triphosphates, such as adenosine triphosphate, which may provide the energy for drug transport. [Source: Michael Gottesman, Igor Roninson, and Ira Pastan]

neck cancers, which usually respond well to therapy with nitrogen mustard drugs, had low concentrations, whereas cancers of the colon, lung, prostate, and pancreas, which are resistant to the nitrogen mustards, had high concentrations.

Cecil Pickett of the Merck Sharp and Dohme Research Laboratories in Rahway, New Jersey, has recently cloned the gene for glutathione transferase and studied its control in normal and precancerous cells. Pickett's work indicates that chemical carcinogens can permanently elevate the expression of the gene in liver cells that are undergoing malignant transformation even though the gene expression remains normal in nearby cells that are not precancerous.

As mentioned, amplification of the P-glycoprotein gene contributes to the development of the classic form of multidrug resistance, although increased activity of the gene may also be involved. According to Pickett, the glutathione transferase gene shows no sign of amplification in the tumors. The increased synthesis of the enzyme results from increased transcription of the gene.

Because many chemotherapeutic drugs act by damaging DNA, cells that have highly active systems for repairing the damage may be more resistant to the agents. Conversely, agents that disrupt DNA repair may potentiate the effects of chemotherapy. For example, Leonard Erickson of the Loyola University Medical Center in Maywood, Illinois, has found that the drug streptozotocin, which inhibits one of the repair enzymes, greatly increases the ability of certain nitrosourea derivatives to kill cultured cells. He has begun clinical trials of streptozotocin and the nitrosourea BCNU, but it is still too early to tell if any of the patients are responding to the combination.

Even though gene amplification may not contribute to increased synthesis of glutathione transferase, it is nonetheless important in the development of resistance to chemotherapeutic drugs. Moreover, several investigators have linked oncogene amplification with the development of highly malignant types of cancers. Recently, Schimke and his colleagues have been focusing on determining how treatment with methotrexate and other chemotherapeutic drugs might lead to gene amplification. They conclude that amplification may be a consequence of the mode of action of many of the agents. "The very process that is leading to the death of the cells is also leading to their resistance to drugs," Schimke says.

Tumor-cell killing by the drugs used for chemotherapy often depends on their ability to prevent DNA synthesis, and as Schimke points out, transient inhibition of DNA synthesis is a common feature of treatments that foster gene amplification. When synthesis of the DNA resumes, it overreplicates, producing extra copies of some DNA segments. Schimke suggests that the overreplication may be the result of the accumulation of proteins that regulate DNA synthesis. He and his colleagues found that increased transcription of the dihydrofolate reductase and other genes occurs during the time when DNA replication is inhibited, with a consequent increase in the production of the corresponding proteins.

According to the model proposed by Schimke, DNA overreplication can lead to the generation of several kinds of chromosomal abnormalities that are often seen in tumor cells. These include broken chromosomes, inversions of chromosome segments, the extrachromosomal fragments called "double minutes" that are associated with drug resistance, and extra duplications of chromosome regions or whole chromosomes.

Drug resistance is the most important and challenging topic in cancer treatment research today.

The results of the studies of how drug resistance develops carry a number of implications for cancer therapy. One of the lessons learned is that prolonged admininstration of low drug doses is a very effective method for producing resistance. Consequently, there is a growing tendency to give patients short-term, but intensive, chemotherapy. "If you are going to make it to cure, you are going to make it early. Resistance will develop later," says Emil Frei of the Dana-Farber Cancer Institute in Boston.

Frei and his colleagues are currently investigating a new, intensive chemotherapy that combines two alkylating agents, drugs that block DNA synthesis by causing cross-links to form between the two strands of the DNA double helix. The therapy represents a departure from the standard forms of combination chemotherapy. Ordinarily, drugs with different mechanisms of actions and side effects are combined, so that the tumor-cell killing activities will be additive while the side effects are not. Combining different types of drugs was also supposed to lessen the chances of the cancers becoming resistant to the therapy.

The Dana-Farber workers decided to combine the alkylating agents because studies with cultured cells had shown that these drugs do not readily induce resistance, and if resistance does develop to one alkylating agent, it usually does not extend to others. Moreover, some combinations of the drugs proved to kill the target cells synergistically.

Frei and his colleagues are now testing a combination of the alkylating agents cyclophosphamide and thio-TEPA in patients with advanced cancer. Although the drugs are administered for only 3 days, the doses are sufficiently high to have lethal effects on the bone marrow. To avoid this, the clinicians remove some of the patients' marrow before giving them the drugs and then restore the bone marrow to the patients after the drug therapy is completed. Frei is encouraged enough by the trial results to begin planning a new study in patients with less advanced disease.

The development of drug resistance in tumor cells does not mean that they have become completely invulnerable. "Leukemias and lymphomas that are resistant to conventional chemotherapy can still respond to high-dose chemotherapy or radiation," say Lee Nadler, who is also at Dana-Farber. However, the doses that can be given are limited by their potentially fatal side effects.

Nadler has been testing a high-dose regimen of drugs and radiation in patients who have the blood-cell cancer called non-Hodgkin's lymphoma and who have relapsed after responding to their initial drug treatments. The new therapy would normally be fatal because of its toxic effects on the bone marrow. Nadler therefore gives the patients transplants of their own bone marrow, which is removed before the intensive drug therapy and treated with a monoclonal antibody to destroy any lingering cancer cells before it is injected back into the patient. This treatment has induced complete remissions in all of the 40 patients treated thus far. About 60% have remained disease-free for up to 4 years.

The patients could also be given transplants of donated marrow, but this runs the risk of causing graft-versus-host disease, in which immune cells produced by the foreign marrow attack the tissues of the recipients with potentially fatal results. However, this possibility can be greatly minimized, according to Nadler's Dana-Farber colleague Jerome Ritz, by treating the marrow before it is transplanted with monoclonal antibodies that are directed against the cells that mediate graft-versus-host disease.

The resistance of many common cancers to drugs has long been a stumbling block to successful chemotherapy. Nevertheless, researchers have been learning a great deal about the versatile repertoire of defenses with which cancer cells ward off the killing effects of chemotherapeutic agents. The information is already pointing the way to better chemotherapies. ■ JEAN L. MARX