Chemoprevention of Prostate Cancer

"A Conversation with Paul Sieber, M.D."

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INTRODUCTION

We've all been taught that screening leads to early detection, which leads to early treatment, which, in a perfect world, leads to a good chance for a cure; right?

Not always.

Take prostate cancer, for example. The more we screen, the more positive reports we get, which may lead to unnecessary treatments, which leads to a good chance of creating some *extremely* undesirable side effects in otherwise healthy men. And as for a cure — once tumors start growing in the prostate, we can't seem to stop most of them from spreading.[1]

Despite such dire predictions, the effects of screening haven't been entirely devastating. For example, the number of men dying from this disease is actually starting to decline, and prostate cancer has fallen from the second leading cause of cancer-related death in men[2] to the third. Unfortunately, the number of men developing the disease for the first time remains high, with well over 200,000 new diagnoses each year and a prediction that this number will continue to rise.[3,4] Additionally, the burden of this disease remains, as always, primarily on older men and African-American men.[3]

How can we catch these tumors earlier and stop their growth? Obviously we can't do anything about age or genetics. And as for more screening — at best, that's "controversial." It looks like our best bet is to find out what triggers tumor formation in the prostate and find a drug that will put it out of action early.

THE SEARCH FOR BIOMOLECULAR TRIGGERS

Several potential markers for prostate cancer have already been identified and are the basis for several ongoing clinical trials. They're quite diverse — increased androgen activity, decreased estrogen activity, an inadequate diet, chromosomal changes in premalignant lesions. But they seem to share two mechanisms of action: oxidative stress and abnormal genetic translation.

PROSTATE ENEMY #1: OXIDATIVE STRESS

Clues to the role of oxidative stress in prostate cancer were found in a couple of nutrition studies: the ATBC (α-Tocopherol and β-Carotene) study, which studied whether vitamins E and A can reduce the risk for lung cancer, and the Nutritional Prevention of Cancer Study, which studied whether selenium reduces the risk for skin cancer.[5,6] They didn't find what they were looking for, but they did find that *all 3 nutrients reduce the incidence of prostate cancer.*[2]

These nutrients share a common mechanism of action: *antioxidant activity.* A shortage of antioxidants (i.e., oxidative stress) leaves free radicals "free" to interact with DNA and promote carcinogenesis. Androgens might contribute to this problem; they've been observed triggering the release of free radicals in human prostate cancer cell lines.[2] This problem could be solved by blocking androgen activity — which means blocking 5-α reductase activity to prevent the conversion of testosterone into dihydrotestosterone, which actually carries out androgen activities.[7] If we can do that effectively, we might just find ourselves on the road to a cure.

PROSTATE ENEMY #2: A BAD TRANSLATION

Changes in gene expression (e.g., to reduce apoptosis) and structure (e.g., increased methylation) may allow normal epithelial prostate tissue to progress to premalignant tissue, then to local adenocarcinoma, and finally to metastatic disease.[2] If we could identify the specific genes or gene products that are affected, perhaps we could use them as markers to stage prostate cancer more accurately.

THE SEARCH FOR TRIGGER BLOCKERS

Four key clinical trials have been designed to evaluate several methods of blocking cancer triggers in the prostate: PCPT (Prostate Cancer Prevention Trial), the REDUCE (Reduction by Dutasteride of Prostate Cancer Events) Study, SELECT (Selenium and Vitamin E Cancer Prevention Trial), and PIN (Prostate Cancer Prevent Study for Men With High-grade Prostatic Intraepithelial Neoplasia [PIN]).

One of Lancaster General's own, Dr. Paul Sieber, has participated in all four studies.

PCPT

PCPT was a 7-year, phase 3, randomized, double-blind, placebo-controlled trial of finasteride (Proscar) — which inhibits type 2 5α-reductase — in 18,882 healthy men aged 55 years and older.[4] This study was terminated 2 years early, when the investigators found that the incidence of prostate cancer had dropped by close to 25% in men taking finasteride.[8] That would mean that 316,760 person-years could be saved with this drug. But don't be too impressed: The finasteride group also had very aggressive tumors (Gleason scores: 7-10), enough to reduce the number of person-years saved by close to 40,000.[9]

Dr. Sieber was not impressed at all by PCPT. Currently chief of the Division of Urology at Lancaster General, Sieber was a "young" doctor when the PCPT team started enrolling men in 1993.[4] But even now, with 85 clinical trials under his belt, he's bothered by the trial's study design. The first problem: the enrollment criteria. Every man had to have a PSA of 3 ng/mL to be enrolled, but digital rectal exams (DREs) and biopsies were not required.[8] Failure to use the DRE is a major concern, because "the physical exam has always been notorious for either underestimating or overestimating prostate cancer," he explained. "What bothered me the most about the study design was that they didn't biopsy everybody when they started." The PCPT team may have assumed that a normal PSA and a normal DRE meant a normal exam. But Dr. Sieber points out that " 'Normal' at that time is now viewed with some skepticism." For example, a PSA reading of 4 to 10 ng/mL was considered a diagnostic "grey zone" in the early 1990s. The current cutoff is 3 ng/mL for everyone preferably tailored to the age of the man.[3] And consider the changes in biopsy technique. "We used to take 6 cores for a biopsy," he explained, "and now we take 12 cores." As a result, the diagnostic rate has increased dramatically. Thus, while using what were state-of-the-art techniques in their time, the PCPT team may have missed a lot of cancer over the years.

This is a drawback for most long-term studies. Techniques can become outmoded before the study ends, making its findings less useful. That's why the REDUCE study was developed—to "correct" some of the shortcomings of PCPT.

REDUCE

The REDUCE study is a 4-year, international, double-blind, placebo-controlled trial of dutasteride (Avodart) 0.5 mg daily in men aged 50 to 75 years.[10] The most obvious improvement in this trial over PCPT is that a biopsy is scheduled every 2 years, in addition to the one required before enrollment. Another important improvement is the "staging" of PSA cutoffs by age: 2.5 to 10 ng/mL for men aged 50 to 60 years and 3.0 to 10 ng/mL for those older than 60.[9]

Yet another improvement is the choice of drug. Dr. Sieber (who is on the advisory board for this study) admits to questioning the advisability of studying a 5-α reductase in PCPT, because at that time this enzyme was thought to be present in the stroma of the prostate and not in glandular tissue, where prostate cancer originates. Given the knowledge base at that time, the idea of studying a 5-α reductase inhibitor seemed like "a joke."

No one's laughing now.

It turns out that 5-α reductase really is found in the glandular tissue, as it's upregulated in prostate cancer. It also turns out there are 2 of them — types 1 and 2.[10] That's why the REDUCE team decided to study dutasteride, which, like finasteride, is a 5-α reductase inhibitor, but unlike finasteride — which targets the only 5-α reductase known at that time (type 2) — dutasteride targets both and, consequently, causes DHT levels to fall must lower than finasteride (90% vs 70%). A good move — since there might be more type 1 5-α reductase in malignant tissue than in benign tissue.[9]

SELECT

SELECT is based on the findings of the 2 failed nutrition studies mentioned previously — ATBC and the Nutritional Prevention of Cancer Study — which revealed that α-tocopherol (vitamin E), β-carotene (vitamin A), and selenium reduce the risk for prostate cancer.[5,6]

SELECT is a 7- to 12-year, multinational, phase 3, randomized, placebo-controlled trial of selenium 200 µg daily, vitamin E 400 IU daily, or combination therapy to see if these supplements can reduce the incidence of prostate cancer.[4] In 2001, this trial started recruiting men aged at least 50 years (African Americans) or 55 years (non-African Americans) (goal: N = 32,400).[4]

Such a straightforward study — what could possibly be wrong with it?

Well, for one thing, PSAs and DREs are not required, except to enter the study. Neither are biopsies. "I'm the urologist of record for our SELECT trial," says Sieber. "I haven't seen a single patient… for a biopsy."

For another thing, SELECT doesn't take baseline selenium readings. Those are difficult to get, anyway, especially for a nutrient like selenium whose levels in soil vary geographically.[6] Second, selenium has a nonlinear relationship with its antioxidative effects[11] — so more is not necessarily better, it might be downright toxic. Third, nutrition studies tend to have the best results in patients who are already malnourished. In fact, the initial "hint" at selenium's cancer-fighting qualities was seen in a study conducted in a part of the world were the availability of fresh fruits and vegetables has historically been low.[4,6] Fourth, it's hard to know how much of a nutrient is already in the diet, unless "you have people living in a lab like a rat and feed them the same meal," quips Sieber.

On the other hand, SELECT has the highest participation of African Americans of any prostate cancer study — 15% compared with the single-digit percentages for total minority enrollment in similar studies.[4] Given that African-American men tend to have the most severe cases of prostate cancer,[3] Sieber applauds their minority recruitment efforts. African-American enrollment is crucial for prostate cancer research. "When you look at blacks and Asians and whites [in terms of] their genetic diversity, whites are among the most monotonous by a long shot, and blacks are the most diverse," he explains. With low minority enrollments, drugs have been designed based on data gathered primarily in whites. Given the genetic diversity of African Americans, Sieber points out that "if you design a drug that seems to work great for whites, it may (only) work for a fraction of black patients."

PIN

The PIN team started recruiting men aged 30 years and older (goal: N = 1260) in 2005 for this 18-month trial of toremifene citrate (Fareston) 20 mg daily versus placebo to evaluate its effectiveness in preventing the progression of PIN to prostate cancer.[12] This drug is currently indicated for postmenopausal women with metastatic breast cancer.[13] In breast tissue, it seems to block estrogen activity — possibly by blocking one of two known estrogen receptors (ERs), specifically ER-alpha.[13] But there's a second ER (ER-beta), which seems to be dominant in the prostate and decreases in concentration during prostate cancer.[14] If this study works, it might be a result of toremifene serving as an agonist at ER-beta, more so than by serving as an antagonist at ER-alpha.

Sieber suggests that the premalignant status of PIN is controversial in the academic world, but in clinical practice, "there's no question that PIN and prostate cancer are associated [with each other]," adding that when he sees cancer on one side of the prostate and PIN on the other, cancer is almost always lurking on the second side.

WHAT'S NEXT?

Dr. Sieber mentioned a few things in passing that might very well become the foci of future studies:

Finding a role for inflammation. Chronic inflammation leads to oxidative stress, chronic inflammation is common in prostate biopsy specimens,[2] and, according to Sieber, "People who are regularly using antiinflammatories have less prostate cancer."

Finding a chemopreventive role for statins. "There's still [some] pretty hot [talk suggesting] that the prostate cancer risk goes down in people who take statins," he claims.

Finding a predictive biomarker. Dr. Sieber mentioned that prostate cancer doesn't have many biomarkers compared, say, to breast cancer. With men living longer, that means a slow-growing prostate tumor can reach a dangerous stage before it is detected. Sieber hopes to see biomarkers that can be used to predict cancer progression in specific patients over specific periods of time. With continued advances in molecular cytogenetic techniques, his wish may soon become reality.

Finding predictive histopathological tests. Finding a biomarker without having a useful way of monitoring seems like a waste of time. Fortunately, several immunohistochemical stains are already available that allow us to do just that. We already know they can be used to trace the loss of proteins that control the cell cycle, such as p27, and obtain prognostic information about cancer. Maybe someday such information can be coordinated with changes in tumor scores and other data to let us predict disease progression over time.

By satisfying this "wish list" and finding positive outcomes from the 4 trials described above, we may finally be able to offer vulnerable patients effective chemoprotection against prostate cancer. Will this happen in your lifetime? To quote Dr. Sieber: "We'll see."

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Advances in Breast Cancer Screening and Diagnosis

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ABSTRACT

Breast cancer is the most common form of cancer in American women and the second leading cause of death. In the continuing battle against this disease, surgeons and radiologists are constantly refining techniques to find the disease early, stage it accurately, and develop evidence-based treatment plans tailored to the individual patient with the intention of preserving the patient's breast and quality of life. This article is a review of recently developed strategies for diagnosing breast cancer, identifying hard-to-reach tumors, minimizing tissue sampling errors, and making an accurate diagnosis, so that the resulting plan of contemporary treatment will include all options and procedures.

This is the first in a series of articles about breast cancer. Future articles will delve into the latest advances in treatment options and other aspects of care for patients with breast cancer.

INTRODUCTION

One out of every seven women in a doctor's care will develop breast cancer, and about 20% of these women will die of the disease.[1] Gone are the days when the only screening program for most women was their own ability to feel a lump in the breast. Also gone are the days when the only treatment option was a radical mastectomy. Since the 1990s, there has been a burgeoning of screening and diagnostic strategies for breast specialists that allow us to find some of the smallest nonpalpable lesions deep in breast tissue, and have dramatically improved our ability to make an accurate diagnosis. Some of these strategies are updated versions of old standards, like mammography. Others involve new applications of standard technology, such as using imaging techniques and increasing the use of ultrasound and the addition of MRI for accurate diagnosis. These strategies allow both preoperative counseling and planning, and dramatically decrease the need for excisional biopsies.

This is a review of state-of-the-art techniques for the screening and diagnosis of breast cancer. The indications—and limitations—of these strategies are presented, and those that are promising but still under investigation will be distinguished from those that are ready for use in the office, the radiology suite, or the OR.

PART I: SCREENING

The breast self examination (BSE) remains the gatekeeper to breast cancer detection, with patients finding the most tumors that result in a diagnosis.[2] In younger women, a lump found on BSE is more likely to be benign. This does not make the BSE any less important in this age group, however, because breast cancers are diagnosed in women as young as their 20's. A regular BSE gives younger women a chance to "get to know what normal is," so they can recognize changes in their breasts quickly and report them to you promptly.

The American Cancer Society (ACS) strongly recommends that we continue to encourage all women to examine their breasts on a regular basis. A clinical breast exam should be conducted at least once a year for women older than 40 years and at least once every 2 years for younger women. The office exam provides an excellent opportunity to teach your patients how to do a BSE or to check their technique. The earlier a woman learns how to do the BSE, the better. At Lancaster General, group classes in BSE or one-to-one tutorials are offered through the Breast Center. Mothers can teach BSE to their daughters when they reach puberty, so that young women will make BSE part of their personal hygienic routine.

MAMMOGRAPHY

Mammography, complemented by the breast exam, has served as the pillar of breast cancer screening for about 3 decades.[3] Currently, screening mammography primarily serves 2 purposes: (1) to find lesions while they're

still small; and (2) to localize lesions for a subsequent stereotactic biopsy (discussed later). Lesions found on a screening mammogram are evaluated by a radiologist and determined to be either probably benign, or malignant.

Recommendations for Mammography Screening

The ACS recommends a baseline mammogram at age 35 years followed by annual or bi-annual mammography for all women beginning at age 40.[4] Regular mammograms are also recommended for women younger than 40 years who have a family history of breast cancer in a first degree relative. This is especially true if the family history includes premenopausal breast cancer, or an established *BRCA1* or *BRCA2* gene mutation. Ultrasound in alternating years can be useful for screening high risk younger patients who have dense breasts.

Mammography: The Technology

Both film and digital mammography are now available. For *film mammography*, which has been available since the 1960s, the breast is exposed to very low-energy x-rays, which scatter throughout breast tissue while emitting photons that are absorbed onto an image receptor to form a conventional latent image. These images are then archived on a recording device. Film mammography provides very good spatial resolution and contrast, and thus is useful for finding nonpalpable pathology and identifying subtle differences among the various types of soft tissue in the breast. Film storage is a problem, however, and if the film is damaged or inadequate, the study must be repeated.

The overall sensitivity of film mammography is about 85%, and it varies with the expertise of the user. It is frequently combined with a computer-aided detection (CAD) program to improve its sensitivity and to reduce reader variability. CAD converts x-ray films into digitized images that are displayed on a (small) computer monitor after having been analyzed with software that seeks patterns suggesting malignancy. CAD is used after the radiologist has already made an initial assessment, thereby serving as a radiological "re-review" of suspicious areas to reduce the risk of missing any abnormalities. Although CAD is most commonly used for screening purposes, radiologists with limited breast mammography experience often find it beneficial in making a diagnosis.

Digital mammography (also known as *full-field digital mammography*) was developed to overcome some of the limitations of film mammography by segregating the methods of image capture, display, and archiving, so that each component of the imaging process can be manipulated separately for maximum effect.[5] A digital image is created when a digital detector captures photons either *indirectly*—using a scintillator to absorb x-rays and emit scintillated light, which is detected by photodiodes—or *directly*, using a photoconductor to capture x-rays and transform them directly into a digital signal. Digital mammography offers 3 key advantages over film: (1) the images, which are stored electronically, can be transmitted over long distances, allowing clinicians in geographically remote areas to consult with distant specialists; (2) the radiologist can manipulate the images to focus on 3-dimensional images of discrete areas of the breast; and (3) the images are not easily degraded, which simplifies storage. Lancaster General Hospital will convert to digital mammography soon.

The DMIST trial[6] (Digital Mammographic Imaging Screening Trial) compared film versus digital mammography in almost 50,000 women. The trial's report in the New England Journal of Medicine concluded that "digital mammography was significantly better than conventional film mammography at detecting breast cancer in young women, premenopausal and perimenopausal women, and women with dense breasts." They added that despite the added cost (1.5 – 4x the cost of film mammography in the DMIST trial, which concluded in 2003), they believe "the significant improvement in accuracy in specific subgroups of women justifies the use of digital mammography in those groups." Since that trial was completed in 2003, improved understanding of the role of *BRCA-1* and *BRCA-2* genes in putting young women at high risk for developing breast cancer suggests that they too might benefit from digital mammography.

ULTRASOUND

Ultrasound, often referred to as "the stethoscope of the breast specialist," is most often used for diagnosis of a mass that has already been located by palpation. Ultrasound can usually identify masses as cystic or solid, and can recognize characteristics of solid masses that are strongly suggestive of malignancy. Sonography is particularly important in young women, whose breast tissue tends to be dense and thus more likely to cause a falsely negative mammogram.[7]

The Technology

Sonography takes advantage of the fact that controlled sound waves reflected off body tissues provide information not only about the distance of the tissue from the sound source, but about its size, shape, and internal consistency (e.g., fluid vs. solid). A transducer is used to transmit high-frequency sound waves into the body, and to record the character and strength of reflected waves to produce a real-time, dynamic image of the target tissue on a computer monitor. Still frames can be generated to allow the radiologist to evaluate and document the appearance of lesions and suspicious areas in the breast.

Ultrasound is very operator dependent. The complexity of breast ultrasound is compounded by the necessity to correlate sonographic findings with the mammogram, so the sonographer should also be experienced in mammography. An operator who is not familiar with breast tissue may fail to position the transducer properly and miss areas in which occult lesions are most likely to be found. Repeat targeted ultrasound is indicated if the physician has a strong clinical suspicion.

Application

The USFDA has not yet approved ultrasound for breast cancer screening, so its two major applications are: (1) to distinguish lesions found on a mammogram as malignant or benign; and (2) to guide core biopsies, whether performed in the Radiology Department or the surgeon's office. It does not do well in detecting microcalcifications in the breast, which are much better visualized by mammography.

MAGNETIC RESONANCE IMAGING

Contrast-enhanced MRI captures 3-dimensional images and detects lesions hidden in dense tissue, making it suitable for finding tumors that mammography misses.[8] It has excellent sensitivity for invasive malignancy, and high grade Ductal Carcinoma In Situ (DCIS). MRI often misses low grade DCIS, but is particularly helpful for diagnosing multifocal DCIS in patients with in situ calcifications, and detecting other malignant lesions that can not be seen on a mammogram.

How an MRI Detects Cancer

MRI detects cancer by evaluating patterns of enhancement in the breast. Tumors, whether benign or malignant, grow their own blood supply ("tumor angiogenesis"); since blood vessels of benign and malignant lesions tend to differ in both organization and permeability, they enhance in different ways under MRI. Additionally, dynamic scanning allows the radiologist to see how a lesion enhances over time.

In addition to the pattern of enhancement, the shape of a lesion is important in determining whether it is benign or malignant. Computer systems such as DynaCAD make it easier to evaluate MRI images by generating contrast enhancement curves and producing 3-dimensional images of the area containing the lesion (J. Kegel, MD personal communication, June 2006).

Indications

The American Society of Breast Surgeons has identified 5 indications for breast MRI:

1. To localize primary occult lesions in patients with axillary metastases.
2. To determine the extent of tumor involvement in the ipsilateral breast and evaluate the contralateral breast in patients with proven cancer.
3. To monitor the response to neoadjuvant chemotherapy.
4. To screen patients with *BRC1* or *BRC2* mutations.
5. To rule out cancer in patients who have an indeterminate physical examination, mammography, or ultrasound.

High-risk Screening

MRI is recommended for women younger than 40 years who have an elevated risk for breast cancer because they harbor a *BRCA1* or *BRCA2* mutation or have several first degree relatives who have had breast cancer. The *BRCA1* and *BRCA2* mutations are found with increased frequency (2.3%) in individuals of Ashkenazi (Eastern European Jewish) descent, as well as in natives of Norway, the Netherlands and Iceland.[9,10] These mutations are thought to interfere with the repair of double-strand breaks in DNA by blocking a nucleotide excision repair pathway that is activated by lesions that distort the helix (e.g., oxidative stress). The altered *BRCA1* gene product is also believed to affect patterns of growth and differentiation in breast epithelial cells, thereby increasing the risk of oncogenesis. LGH works closely with screening programs of the University of Pennsylvania and Hershey Medical Center to refer appropriate patients for genetic screening. Unfortunately, insurance coverage for the $3,000 cost of genetic screening is often denied.

PART II: DIAGNOSIS

INTRODUCTION

The key to an accurate diagnosis is an adequate amount of relevant tissue for cytological analysis. Before the 1990s, a suspicious lesion was often excised completely for histological examination. In an early attempt to reduce the size of the biopsy, technology first swung to the opposite extreme by developing fine needle aspiration biopsy (FNAB), in which thin (25-gauge) needles were inserted and an area was sampled under the guidance of either palpation or ultrasound. FNAB reduced scarring and the trauma of a biopsy, but often failed to obtain enough tissue for an unequivocal diagnosis.

Biopsy techniques have evolved over the last two decades, with development of hollow needles that are just wide enough to obtain an adequate sample size, and are guided by advanced imaging techniques to minimize tissue sampling errors. The following section discusses these minimally invasive needle biopsy procedures, which have virtually replaced excisional biopsy.[11,12,13]

Core Needle Biopsy

Core needle biopsy is used under ultrasound, mammographic, or MR guidance to remove solid cylinders of tissue, using a relatively wide (10- to 14-gauge) hollow needle attached to a tissue sampling device. The device may be spring-loaded or vacuum suction assisted, or it may employ a cryo-assisted core gun.

Spring-loaded devices operate almost like a gun. A notched needle is mounted onto a spring-loaded device and "shot" into the target tissue, where a small cylinder of tissue is cut out and collected in the notch. When used to diagnose in situ and invasive cancers, this technique very rarely produces false-negative results. Preop diagnosis by core biopsy reduces re-lumpectomy rates and most importantly preserves the surgeon's ability to perform sentinel lymph node biopsy (SLNB) for axillary LN staging.

Vacuum-assisted biopsy—e.g., the Mammotome probe (Biophys, Irvine, Calif)—is one of the most accurate methods of determining the histology of microcalcifications by taking multiple core samples in an area.

The cryo-assisted rotational device (Sanarus, Cassi) uses a freezing process that minimizes bleeding and trauma.

No matter which technique is used, the core biopsy site is marked, typically with a titanium clip. If the results of core biopsy are benign, the clip serves as a marker on subsequent mammograms. If the biopsy results are malignant, the clip serves as a marker for mammographic or ultrasound guided needle localization on the day of surgery. A specimen x-ray is typically obtained at the time of surgery to confirm that the tissue removed contains the lesion/microcalcifications and clip. In some rare cases, the core biopsy does not produce a definitive diagnosis and an excisional biopsy is necessary.

Stereotactic Needle Biopsy

Stereotactic needle biopsy is predominantly used to obtain a histologic diagnosis of microcalcifications, but it can also be used to biopsy areas with architectural distortion and masses which can not be seen sonographically. X-rays taken from two different angles provide stereo images of the biopsy path, which then create a 3-dimensional image of the area of interest and improve the accuracy of needle placement.[1] The images are taken with the patient lying prone on a specially constructed table that allows the breast to hang down through an opening. The radiologist or surgeon and x-ray technologist sit below the table and take several pairs of images. Key coordinates are then identified on a computer monitor, and small core tissue samples are removed by vacuum assisted needle biopsy.

This useful technique does have some limitations, and other techniques are sometimes substituted. The necessary positioning is not suitable for patients with severe neck or back problems, patients who weigh more than the table can accommodate (approximately 250 lbs), and those who are extremely anxious (though pre-procedure sedation with Valium is very helpful).

Percutaneous Excisional Biopsy

Devices are available that excise small lesions entirely. Currently, these are not used at LGH because current core techniques are accurate and very small benign lesions can be left in situ and followed with radiology studies.

SUMMARY

State-of-the-art options for screening and diagnosis have improved our ability to detect and diagnose breast cancer early, but the BSE and mammogram remain the mainstays for detection of breast cancer. Mammography, particularly with computer-aided detection, can now produce

images detailed enough for diagnostic purposes, and digital mammography allows transmission of 3-dimensional images over long distances. These advances mean that we are close to removing major logistical barriers to access to screening and care for breast cancer.

MRI has an increasingly important role in breast imaging, especially in high risk patients, and those with an indeterminate physical exam, mammogram, or ultrasound. It is particularly useful in the search for occult disease, and to see if a recognized tumor is more extensive than seen on mammography. Ultrasound is an essential complement to breast imaging, particularly when a lump is palpable, or in young women whose breasts appear dense on mammography. Breast biopsy techniques have improved dramatically over the past two decades, and image guided core biopsy techniques have essentially eliminated the need for excisional biopsy to obtain a diagnosis. Ultrasound is the mainstay of guidance for core biopsies.

Ultrasound and MRI now help provide the information needed to develop treatment plans that are exquisitely tailored to each patient, and dramatically increase the chances of saving every woman's breast, as well as her life.

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CCFA:
MD ACCOMPLISHMENTS

HANAUER, STEPHEN B

Dr. Hanauer received funds from CCFA from 1992 to 1995 to carry out a multicenter evaluation of the efficacy of methotrexate in chronically active CD.

Methotrexate has been proven effective in moderate to severe CD (1) and to maintain remission in adults with CD (1,2). Hanauer participated in several studies evaluating its efficacy and safety, particularly in maintaining remission.

In a double-blind, placebo-controlled, multicenter trial in patients with active CD who had entered remission, the investigators found that a significant number of these patients were able to remain in remission long-term (40 wk) on a reduced dose (15 mg IM vs 25 mg IM once weekly), and significantly few needed prednisone because of relapse compared with placebo (3).

In a study of the adverse effects of IBD drugs, the investigators found that methotrexate carries a range of adverse effects, including nausea, leucopenia, and, rarely, hepatic fibrosis or hypersensitivity pneumonia (4). It is also contraindicated in pregnancy (5). Because of concerns over hepatotoxicity, a study was designed to determine whether surveillance liver biopsies are warranted in IBD (6). The patients (N=20) had experienced long-term methotrexate therapy. Liver biopsies revealed only mild histological abnormalities (Roenigk's grade I and II) and one case of hepatic fibrosis. Abnormal liver chemistry test results were seen in 30% of patients, none of whom demonstrated Roenigk's grade IIIB hepatotoxicity. The investigators concluded that surveillance liver biopsies were not warranted for such patients. Because of its adverse effects, however, this agent is considered for second-line therapy in patients who are refractory to or cannot tolerate 6-MP/azathioprine (1).

Hanauer also helped evaluate the steroid-sparing effect of methotrexate in CD in a study of patients (N=76) with long-term CD (mean: 9.5 y) and methotrexate therapy (mean: 55 wk; mean dose:20 mg/wk). Improvement was seen in 63% after 9 weeks of therapy and lasted 65 weeks, while remission was seen in 37% after 22 weeks of therapy and lasted 59 weeks. The results were best with parenteral therapy and in younger patients (<40 y) (7).

Continued evaluation of this drug is warranted, given the fact that drugs in this category have already demonstrated their potential for extending the duration of infliximab therapy by keeping the level of infliximab antibodies relatively low (8). This effect, if seen in methotrexate, may indicate the potential for combination therapy.

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KORELITZ, BURTON I, MD

Korelitz received funds from CCFA from 1993 through 1995 to develop a double-blind, randomized trial of 6-MP versus 5 aminosalicylic acid in the prevention of recurrent ileitis after resection in patients with CD.

Judge and Lichtenstein cited studies in which Korelitz participated to indicate that complete 6-MP may be helpful for achieving fistula closure (1) or to complete fistula healing and remission (2). These studies were also used to identify any serious adverse events that can result from 6-MP therapy (3).

Markowitz (4,5) and Dubinsky (6) cited studies by Korelitz indicating that azathioprine and 6-MP are efficacious in patients with CD who develop fistulas. Markowitz also cited studies by Korelitz and colleagues providing anecdotal and trial evidence that 6-MP reduces the rate of postsurgical endoscopic (6 mo) and clinical (12 mo) relapse.

The results of a 1993 study of mesalamine monotherapy in patients intolerant of the parent drug (sulfasalazine), in which Korelitz participated, indicated that the drug was effective in both CD and UC, and that it was more effective than the parent drug in CD (7).

Korelitz participated in a 2-year study comparing 6-MP, 5-aminosalicylic acid, and placebo in preventing the postoperative recurrence of CD (8). The results, reported in 2004, indicated that the recurrence rate was lowest with 6-MP compared with mesalamine and placebo, whether recurrence was evaluated clinically (50%, 58%, and 77%, respectively), endoscopically (43%, 63%, and 64%, respectively), or radiographically (33%, 46%, and 49%, respectively).

Thus, although both agents are safe and effective in CD, Korelitz demonstrated that combination therapy may preclude the need for additional surgery in CD patients with fistulas.

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TARGAN, SR

Targan received funds from CCFA from 1981 to 1982 to study the cytotoxicity of natural killer (NK) cells in normal and IBD intestinal mucosa.

In an early study by Targan and other investigators (1), two systems of antibody (antitetanus toxoid) suppression, one of which appeared to be mediated by NK cells. Shortly thereafter, Deem and Targan (2) delineated the sequence in which an NK-derived cytolytic factor (NKCF) induces cytolysis that indicated that this process is strongly influenced by the presence of gluteraldehyde.

Shortly thereafter, a Targan team found that 6-mercaptopurine (6-MP) could inhibit NK-cell cytolytic activity in patients with CD. Until then, spontaneous cytotoxic activity had not been observed in the human gut. Another Targan team, after identifying NK-positive lymphocytes within the lamina propria of the gut (4), proposed that such activity might not have been recognized or linked with NK-cell activity previously because NK cells in the gut are phenotypically different from those in the peripheral blood.

The cytolytic activity of NK cells was clarified further by 1987, when Targan and colleagues demonstrated that phospholipase A2 (PA2) inhibitors also inhibited NK-mediated cytotoxicity. They suggested that PA2 may also modulate the surface of NK cell targets to uncover a secondary "trigger" that facilitates cytolytic activity (5). Eventually PA2 activity was found to correlate with tumor necrosis factor (TNF)-alpha activity, such that TNF expression was apparently activated by PA2 (6), TNF apparently triggered PA2 activity (7), and substances that inhibited PA2 activity apparently also blocked TNF activity in a dose-dependent manner (8).

In 1997, a Targan team investigated the role of a TNF-alpha antibody in patients with CD (9, 10). This antibody—a chimeric monoclonal antibody known as cA2—was given to 108 patients with moderate to severe CD in a 12-week multicenter, double-blind, placebo controlled trial. A 61% clinical response was seen by week 2 and remained significantly greater than the response in the placebo group throughout the study. By week 4, a third of the active treatment patients were in remission.

This antibody is currently formulated as infliximab (Remicade®), which is now indicated for moderate to severe CD and for fistulizing CD (11).

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MARKOWITZ, JAMES F, MD

Dr. Markowitz received funds from CCFA from 1991 through 1994 to develop a prospective, double-blind, multicenter, placebo-controlled trial of 6-mercaptopurine and corticosteroids in children and adolescents with newly diagnosed CD.

Prior to the start of this project, the long-term efficacy of 6-MP in adolescents with intractable CD was not clearly established. Markowitz and colleagues conducted a study in adolescents (N=36) who had been taking 6-MP for at least 6 months and had been intractable to other IBD agents, antibiotics, and nutrition support for approximately 5 years before starting 6-MP therapy. During the first year of treatment, patients exhibited a higher Lloyd-Still disease activity score and improvements in physical exam, nutrition, laboratory tests, and general activity scores. Annual hospitalization rates also declined (1).

In 2000, the Markowitz team conducted the first controlled trial of 6-MP/prednisone combination therapy versus prednisone monotherapy in children with steroid-dependent CD. They found that 6-MP significantly reduced the need for prednisone and prolonged the duration of remission (3). The adverse events were similar to those seen in adults, including the increased risk for cancer. Concern over this and other adverse effects may be avoided in the future using metabolite tests (the thiopurine methyltransferase genotype/phenotype test and the 6-MP metabolite test) to optimize therapy, detect noncompliance, and reduce the risk for toxicity associated with this drug (4,5).

Despite the apparent efficacy and safety of this drug, continued evaluation is warranted, given the recent controversy over the management of CD in children. American pediatric gastroenterologists appear to be comfortable prescribing immunomodulator drugs for children younger than 5 years (6) and prefer to start therapy in children with steroids and azathioprine (the parent drug of 6-MP), their Western European counterparts prefer to start with nutrition therapy before progressing to budesonide or steroids, at least in children with mild to moderate disease (7). Continued discussions in this area may help the physicians worldwide to reach a consensus.

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ROTTER, JEROME I, MD

Dr. Rotter received CCFA funding from 1992 through 1994 to investigate the role of molecularly defined HLA class II genes in IBD.

At that time, few studies of HLA class II genes in patients with CD or UC were available (1). Those that were available had been carried out using serological techniques and had inconclusive results. When those techniques were replaced with molecular genotyping and allele-specific oligonucleotide hybridization, the investigators discovered a positive relationship between the HLA DR2 allele and UC and a positive association with the HLA DR1 and HLA DQw5 alleles and CD.

Previously, it had been observed that antineutrophil cytoplasmic antibodies (ANCAs) are also associated with UC (3), suggesting a disturbance in immune regulation in UC (2). The investigators in that study also found that patients with UC were likely to demonstrate a link between ANCA and DR2, which suggests that a subset of UC patients may be genetically susceptible to an immune defect that serves as the basis for the disease (2).

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SARTOR, RB

Dr. Sartor was funded by the CCFA from 1987 through 1989 to study the role of bacterial cell walls in the pathogenesis of CD.

The potential contribution of macromolecules crossing normal and injured intestinal tissues to intestinal inflammation had been discussed previously (1-3). Peptidoglycan-polysaccharide (PG-PS) complexes within bacterial cell walls had also been recognized as being responsible for the outcome of inflammation and immunomodulation in granulomas that follow bacterial infection, but their uptake across the intestinal epithelium had not been investigated.

The Sartor team decided to investigate this phenomenon by studying rats in which the induction of colonic injury was followed by injection into the cecum of a small amount of ^{125}I-labeled purified PG-PS fragments obtained from Group A *Streptococcus pyogenes* organisms (4). The results were dramatic: all of the rats developed signs of illness within 24 hours. Illness was indicated by gross evidence (surface hemorrhages near the site of injection and on focal areas of the transverse and descending colon and rectum), microscopic evidence (eg, marked thickening of the lamina propria and dense PMN infiltration), and systemic distribution, indicated by elevated levels of radioactivity in the liver, spleen and mesenteric lymph nodes. Based on these findings, the investigators suggested that PG-PS derived from the normal enteric flora may induce or sustain inflammation within the intestines and in extraintestinal tissue in patients with CD or UC.

Evidence of the systemic spread of PG-PS-induced intestinal inflammation was supported further by a subsequent study of rats in which intestinal injury was induced in the jejunum by means of a surgically created blind loop, within which a proliferation of anaerobic bacteria occurred. The results of these two tests may not be completely equivalent, because immunoreactivity was measured in this study by serological and histological tests, only. However, histological evidence of inflammation within the lamina propria, hypertrophy of the muscle layers of the gut lining, and measured changes in luminal PG-PS and anti-PG antibodies for 3 classes of immunoglobulins (IgG, IgM, and IgA, whose plasma levels did not change) strongly implicate PG-PS as the inducer of the inflammatory process (5).

Based on this evidence and evidence from subsequent studies of the role of bacteria in intestinal inflammation, Sartor has recommended that the goals of IBD therapy include reduced exposure to luminal bacterial antigens (ie, antibiotic therapy) and correction of the abnormal immune response to gut antigens (6). Antibiotics have generally been reserved for infectious complications of IBD rather than as a component of the primary treatment regimen (7). Currently, metronidazole and ciprofloxacin are frequently used (8), despite a lack of rigorous trials (8,9) or evidence of significant benefit over placebo or sulfasalazine (8). Sartor has suggested that evidence from rodent studies provide a rationale for treating human IBD with antibiotics (7). The evidence provided by his CCFA-funded research may have paved the way for developing a rationale for rigorous controlled trials of antibiotics to ensure that they can be safely and effectively incorporated into standard primary therapy for IBD.

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ELSON, CHARLES O, MD

Dr. Elson was funded by the CCFA from 1981 through 1983 to investigate T-cell regulation of immunoglobulin synthesis in IBD.

In an early study, the Elson team sought to determine whether patients with CD have a defect in immune regulation by evaluating suppressor T-cell activity in patients with mild or inactive disease (1). Their in vitro studies indicated that these patients do not have a deficiency in suppressor T cells; indeed, the suppressor T-cell population markedly inhibited IgM synthesis.

Shortly thereafter, the Elson team published a report of their in vitro study of T cell activity during a mixed lymphocyte reaction (2). They found that T cells that had been stimulated by B cells or macrophages were able to suppress proliferation and immunoglobulin synthesis. The B-cell or macrophage-stimulated T-cell activity observed here led the investigators to believe they had stumbled upon a negative feedback mechanism involved in regulating the immune response.

The next step would be to determine whether these early findings—which were performed on elements obtained from peripheral blood—would also be observed in gut tissue. The Elson team evaluated the T-cell immune regulatory effects (suppression or "help") in T cells obtained from intestinal lamina propria tissue that was isolated from patients with CD (3). As in the previous two studies, immunoglobulin synthesis was stimulated by adding pokeweed mitogen to the culture. Additionally, helper T-cell activity was elicited by adding normal peripheral blood cells to the cultures containing lamina propria T cells, and suppressor T-cell activity was elicited by adding B cells to cultures containing irradiated normal T cells (x-irradiation eliminated suppressor T-cell activity in an earlier study [2]). Suppressor T-cell activity was not significant in any of the cocultures, whether the cells were obtained from healthy controls or activity inflamed CD tissue. The investigators concluded that T-cell immune regulatory activity in the gut is carried out primarily by helper T cells, rather than by suppressor T cells, as was seen in the peripheral blood.

Finding that the immune response in gut tissue may allow for immunoglobulin production led Elson to speculate about the possibility of developing an intestinal vaccine (4). The basic requirements for an enteric vaccine are (a) the ability to trigger the production of adequate amounts of intestinal IgA antibodies; (b) the use of antigens that can induce neutralizing antibodies, and (c) the use of an effective antigen delivery system (5).

In their search for the types of helper T cells and cytokines involved in antigen uptake and presentation in the gut, Elson and colleagues found two helper T-cell subsets: Th1 cells—which are involved mainly in cell-mediated immunity and help produce IL-2, IFN-gamma, and TNF-beta—and Th2 cells, which regulate and promote B-cell responses and help produce several interleukins, including IL-5, IL-6, both of which trigger surface B cells to secrete IgA. (6). They also discovered that the GI lamina propria has a relatively high concentration of IL-5-producing Th2 cells and that these cells are stimulated primarily though the oral route (as opposed to Th1 cells, which are stimulated primarily through the systemic route). Additionally, time-course and dose-response studies during this trial indicated that responses to antibody exposure develop according to different sets of kinetics for oral versus serum routes of administration (7).

This led investigators to look for the most effective route of administration. When tetanus toxin (TT) was administered through an indwelling intraperitoneal catheter, high levels of anti-TT antibody-secreting cells were detected in the general circulation and the peritoneal cavity. The predominant immunoglobulin elicited was IgG (80%), rather than the more critically important IgA. Additionally, TT failed to elicit a salivary response. Given that an intestinal antigen is likely to reach the gut by the oral route, the inability to elicit an antibody response in the oral cavity put the patient at risk for prolonged inflammation (8).

Elson was on the first research team to find evidence of a circulation route by which newly activated antigen-specific intestinal T cells return to the gut (10). They also found that activated T cells complete this circuit--through mesenteric lymph nodes, the lymphatics, and blood—and return to the gut guided by cell surface homing receptors (particularly alpha4beta7-integrin). They also discovered that the site of antigen presentation determined whether such homing receptors are expressed or not.

Thereafter, several other investigators searched for a delivery system that could allow oral agents to "survive" the destructive nature of the GI tract, reach the mucosa, and remain there long enough to take effect. Lavelle proposed the use of bioadhesive molecules (eg, lectins) that can recognize epithelial cell surface receptors and thus reach specific regions of the gut (11). Clark and others suggested synthetic delivery particles that can interact with antigen-sampling M cells (12). Zho and Neutra (13) suggested using liposomes (which are quite effective at inducing mucosal IgA responses) that have been modified to withstand the harsh intestinal environment and still interact with M cells. Lo indicated that some investigators are currently looking for genes that might help determine mucosal immunity and thus ensure that immune responses are directed against pathogen-associated targets, only (14). Gene expression studies have led to the discovery of novel receptors of unknown function on the apical membrane of M cells within Peyer's patches. Ligands that are known to trigger pathways used by certain pathogens to invade the intestinal wall are being used to determine the functions of the novel receptors. These ligands may eventually serve as models for developing antigen-loaded nanoparticles capable of binding at these sites to neutralize specific antigens (15). Furthermore, continued investigation of proinflammatory cytokine activity may pave the way to developing a vaccination that takes advantage of host defenses to block TNF-alpha activity and thus modulate the immune response to the bacterial flora in the gut (16).

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CHO, JUDY H, MD

Dr. Cho received funding from CCFA from 1997 through 1998 to conduct genetic mapping studies in IBD.

A substantial amount of epidemiological data had been collected prior to that time suggesting that genetic susceptibility contributes to the development of IBD (1,2). This inspired several investigators to search for specific chromosomal loci that confer IBD susceptibility:

- Mirza and colleagues published the results of their gene-mapping studies, through which they found a CD susceptibility gene (*IBD1*) on chromosome 16 and evidence that this gene may also contribute to UC susceptibility (2).
- Duerr and colleagues (3) attempted to find a link between IBD and chromosome 12, as was done in a British genome screen. They selected 122 white American families that included 208 IBD-affected relative pairs. Given the small sets of affected relatives (ie, relative pairs), they carried out a nonparametric analysis (useful when there is a relative lack of evidence for a Mendelian inheritance pattern or when several genes of low to moderate penetrance may be involved [4]) and a transmission/disequilibrium test (TDT; useful when multi-allelic markers are present [5]) to confirm the British findings.
- Curran and colleagues (6) performed nonparametric analyses of data gathered from a large group of independent European families to demonstrate a link to IBD on chromosomes 12 and 16.
- Neurath and colleagues (7) observed high levels of the transcription factor NK-kappa B in lamina propria macrophages of patients with CD or UC, as well as the consequent increase in the production of several proinflammatory cytokines (IL-1, IL-6, and TNF-alpha) as well as the protein known as p65. They also noted that a specific antisense molecule can downregulate p65 to significantly reduce the production of these cytokines in IBD. These findings suggest the possibility of a molecular approach to patients with IBD.
- Hugot and Thomas (8) reported the findings of several groups who used genome screens (9) not only to confirm the links between IBD and chromosomes 12 and 16, but also to identify additional potential links between IBD and chromosomes 1, 3, 4, 7, 11, 15, and X.
- Hampe and colleagues (10) used a genome-wide search for susceptibility loci to confirm the previously identified link between IBD and 7 chromosomes and to identify 3 additional chromosomes (6, 10, and 22) that might contain genes that predispose individuals to this disease. Of particular interest were the links to chromosome 6p, which suggest an association with human leukocyte antigen and TNF genes, and the suggestion that the link with the X chromosome, which suggests an association with the Ullrich-Turner syndrome.

Others have sought specific genes that confer genetic susceptibility to IBD, including the following:

- Parkes and associates applied the TDT (5) and affected sib-pair test (5) to data from 198 pairs of siblings with IBD and determined that the gene encoding IL-2 may contribute to UC susceptibility (11)

- Noting that an imbalance between IL-1 beta (IL-1 beta) and the IL-1 receptor antagonist (IL-1ra) may play a role in the pathogenesis of IBD, Stokkers and colleagues (12) studied allelic frequencies for IL-1 beta and IL-1ra genes in patients with IBD to determine whether there was a relationship between allelic variants and cytokine production. They found a relationship between UC and several infrequent alleles (Taq1 and Mwo1), but the pathogenicity of this finding was not clear.
- The Stokkers team (13) also studied the role of HLA class II genes in IBD, because the products of these genes play important roles in the immune response. A literature search revealed that UC and CD are each associated with specific HLA class II phenotypes. Additional research may reveal the contribution of these genes to IBD susceptibility.

The search for specific genes responsible for IBD susceptibility expanded exponentially over the following years. By 2001, researchers had identified a strong candidate within chromosome 16—*NOD2.* The gene product normally activates the transcription factor NK-kappa B, thereby allowing the cell to respond to bacterial lipopolysaccharides. Using TDT (5) and case-control analysis, the Ogura team (of which Dr. Cho was a member) determined that same year that *NOD2* undergoes a frameshift mutation through a cytosine insertion in patients with CD. These findings suggest that NOD2 plays a crucial role in CD susceptibility and suggest that a relationship exists between an innate immune response and components of the bacterial cell wall that contribute to this disease (14). In 2003, Cho indicated that a gene on chromosome 16 codes for NOD2/CARD15, a protein that is involved in the immune response to bacterial infection (16), and that three mutations of that gene appear to be independently associated with CD, collectively conferring a 15% to 20% risk for familial CD. Cho also indicated that NOD2 presents an increased risk for ileal disease and an earlier age of disease onset. Subsequently, Drs Cho and Dr Bonen indicated that NOD2/CARD15 is expressed on peripheral blood monocytes (17). They also indicated that three polymorphisms within the gene for this protein complex contain the code for CD, especially in individuals of European descent. Having one copy of a risk allele increases the risk for CD 2- to 4-fold in these individuals; having two copies increases the risk 20- to 40-fold. As a member of the Brant research team (18), Dr Cho helped determine that carrying two of these mutations increased the risk for early onset of disease, ileal involvement, and the development of strictures or non-perianal fistulas. As part of the Ogura team, Cho was part of the effort to find a link between NOD2 mutations and ileal disease. That laboratory developed a monoclonal antibody against NOD2, then used it to detect NOD2 expression in terminal ileal Paneth cells, specifically in the cytosol near granules that contained antimicrobial peptides, and in the epithelial layer of ileal villi and the colon. This protein was found in both patients and controls and, thus, may help regulate Paneth cell-mediated responses to intestinal bacteria (19).

Dr Cho has continued to explore the genetic mechanisms for IBD susceptibility. In 2004, she identified post-transcriptional dependence of IL-1 beta on NOD2/CARD15 suggesting that a signaling defect may be the underlying cause of pathogenesis in CD (20). She also explored several new genes believed to contribute to CD. Her work may bring investigators closer to identifying the earliest pathways involved in IBD pathogenesis which, in turn, may reveal potential novel therapeutic targets.

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DAS KM

Dr. Das was funded to study the pathogenesis of IBD (1981-1983) and the immunopathogenesis of UC (1983-1988).

During the first funding period, Dr. Das worked with Dr. Nagai to clarify the role of a disease-specific colonic tissue-bound antibody (CCA) they identified previously in patients with UC (1). Specifically they improved their methods of extracting and purifying intact CCA-IgG. They then demonstrated that this molecule binds to colonic mucosal tissue in UC but not in CD or in normal colonic tissue from patients with carcinoma (2).

Das and another colleague, Dr. Takahashi, then characterized a colonic protein that is recognized by CCA-IgG. Using immunorecognition studies, affinity-column chromatography, transblot analysis, electrophoresis, and an iodinated CCA-IgG probe on a variety of tissues obtained from patients with UC, Crohn's colitis, or myeloma, they found CCA-IgG was consistently bonded to a 40-kD protein in colon tissue extracts. This bond was found most frequently in tissue obtained from patients with symptomatic UC and never in colonic tissue obtained from patients with Crohn's colitis (3). These findings suggest the existence of an organ-specific colonic "autoantigen" that might be able to initiate an IgG antibody response in patients with UC.

To learn more about this molecule, Das and colleagues developed monoclonal antibodies against it. Antibody studies allowed them to localize the antibody-40kD antigen interaction exclusively to colonic epithelial cells, specifically within the crypt and on the luminal surface of the epithelium in this study (4), primarily along the basolateral surfaces in a murine study (5), and in both membrane regions in another study in human tissue (6). Such studies also allowed these investigators to discover that

- This antibody-antigen interaction occurred more frequently in colon tumor cells and that its frequency was not affected by interferon gamma (IFN-gamma) (7)
- The 40 kDa molecule is involved in antibody-dependent cellular cytotoxicity (ADCC) against colon cancer cells by UC serum (8)
- 40 kDa expression in colonic cells is accompanied by the expression of intercellular adhesion (ICAM) molecules (especially ICAM-1), which may be involved in the localization of leukocytes to the colonic epithelium during UC (9)

Years after the CCFA funding ended, the investigators continued their investigation of the 40 kDa molecule in UC:

- They gave it a name: P40
- They discovered that it can be found in the goblet cells of normal ileal and proximal colonic tissue, as well as in enterocytes, where its concentration increases in a distal direction (11)
- They found it in several noncolonic areas, including the gall bladder, major bile ducts, fallopian tubes, and epidermis (11), as well as nonpigmented ciliary epithelial cells and chondrocytes (12)—all of which suggests potential areas for extraintestinal complications of UC
- They discovered that P40 is a member of the tropomyosin family (10). The most common tropomyosin isoform found in the intestine—human tropomyosin (hTM) isoform 5 (hTM5) (13)—is an intracellular protein that can be externalized in the colonic epithelium but not in the small intestine(14). hTM5 has such a strong

association with a membrane-bound colon epithelial protein that is suspected of being involved in its transport to the cell surface and may serve as a target autoantigen in UC (14)

- They named the anti-P40 antibody—mAb Das-1(15)—and found that it reacts with liver tissue and is expressed in correlation with the expression of specific liver molecules, including glycogen (15)
- They found that B cells in the lamina propria produce IgG against hTM5, most notably in patients with UC (16)
- They identified hTM5 as a colon epithelial cell antigen that can trigger a significant T-cell response in UC (17)

Thus far, Das and colleagues have produced a substantial amount of information about several components of the autoimmune activity in UC. Additional studies of the antibody and its target (as well as the cellular components responsible for its upregulation and presentation on the cell surface) are needed to bring these investigators closer to finding an effective immunologic approach to therapy.

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DUERR, RICHARD H, MD

Dr. Duerr received funding from CCFA from 2001 through 2002 to study linkage disequilibrium patterns with a novel IBD locus on chromosome 3P.

Chromosome 3p was first identified as being likely to contain IBD susceptibility genes in 1996 (1). Genetic screening of a total of 186 sibling pairs affected with CD and UC provided evidence of a link between IBD and 46 microsatellite markers, with 16 of the strongest markers seen in chromosomes 2, 3, 7, 12, and 15. The strongest linkage to a single marker was identified in chromosome 12. By contrast, chromosome 3 contained several markers that lie adjacent to regions containing genes that code for UC complications, eg, carcinoma of the colon and renal cell carcinoma (1), as well as two autoimmune disorders—MS and inflammatory arthritis—which suggests one or more genes involved in the inflammatory response (2). One chromosome 3p marker--D3S1076--lies near several potential IBD susceptibility genes, including the genes for cytokine receptors 2 and 5, both of which play an important role in immunoregulation (2). Neither of these receptor genes appears to play a role in the IBD phenotype, but they both reside near other potential IBD-related genes, including the gene for lactotransferrin (which may play a role in neutrophil and antibacterial activity), the ubiquitin complex (which may be involved in antigen processing), the cathelicidin antimicrobial peptide and the TRAF interacting protein (which play a key role in TNF-alpha signal transduction), the mitogen-activated protein kinase that is activated by protein kinase 3, and the IFN-alpha receptor (receptor 2).

Being closely associated with so many genes creates a serious research-related challenge, however, because it presents the risk of disease-associated disequilibrium (2), ie, the observed frequency of haplotypes may not agree with the frequency predicted by multiplying the frequency of individual markers within each haplotype. Identification of links between markers and disease requires linkage analysis (3), a procedure that is used to determine the distance between the marker and the susceptibility gene. It involves an investigation of pedigree, usually by identifying families with affected sibling pairs or affected relative pairs; genotyping by polymorphisms the entire genome or certain chromosomes; and determining the approximate position of the susceptibility gene within the genome map, which involves calculating the LOD score (a non-parametric measure of distance between the susceptibility gene and marker) for several points. Linkage analysis is then followed by an association analysis of candidate genes (3).

By 2002, Duerr and colleagues were able to report their finding of a specific IBD locus on chromosome 3p26. Evidence of linkage was set at an LOD score of 2 or more in a previous study (4); in the Duerr study, a LOD score of 3.69 was achieved for D3S1297, indicating a strong linkage between marker and disease (5).

A recent study indicates that more than 20 genomic regions have been identified as containing IBD susceptibility loci (6). Continued work in this area may facilitate the development of genetic strategies for preventing or treating this disease.

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CHANG EUGENE B, MD

Dr. Chang received funding from CCFA from 1999 through 2002 to study barrier defects in IBD.

During this period, Dr Chang's team produced numerous reports of their work on epithelial ion exchange in the intestines. Chang was among the first to characterize the intestinal Na^+/H^+ exchanger (NHE) in intestinal tissue (specifically, an intestinal villus-like subclone [C2bbe]) rather than in nonepithelial mutated fibroblasts (as had been the practice until 1999) (1). To measure NHE activity, he monitored the unidirectional apical uptake of $^{22}Na^+$ under basal, non-acid conditions. This approach represented a dramatic change in the process of evaluating NHE activity. Previously, it was evaluated by monitoring intracellular pH, which can only approximate NHE activity and may be altered by buffers and non-NHE contributions to pH. Thus, Chang and colleagues developed a method that could significantly improve the accuracy of research findings. Using this improved technique, the Chang team found that the brush-border NHEs—NHE2 and NHE3—both localize to the C2bbe apical domain. They also found that both NHEs are regulated by second messengers, albeit through different signal transduction pathways.

The precise characterization of such exchange molecules will prove essential in determining the role of anion secretion in IBD, either as a participant in complications (eg, diarrhea) or as a regulatory signal. For example, the Chang team found that an oxidant (monochloramine) could potentiate colonic calcium- and cAMP-stimulated chloride ion secretion through its effect on calcium-activated potassium channel conductance. This could increase the severity of diarrhea in patients with an inflamed colonic mucosa (2). They also found that short-chain fatty acids—produced by fermentation of dietary carbohydrates carried out by the bacterial flora in the colon—enhance apical NHE3 activity (but not NHE2 activity) in a time- and concentration-dependent manner and, thus, may serve as a physiological cue that allows the colon to adjust its sodium absorption rate in response to ongoing changes in dietary carbohydrate and sodium loads (3).

Another Chang team investigated the effect of IFN-gamma on ion transport across the intestinal epithelium (4). This could be a critical component of the pathogenesis of IBD, because IFN-gamma helps regulate and promote B-cell responses and helps produce several interleukins that facilitate B-cell secretion of IgA. Chang and colleagues assessed Na^+/K^+-ATPase activity using the inhibitor ouabain and monitored intracellular Na^+ with the Na^+ ionophore monensin. They found that IFN-gamma acutely reduced Na^+/K^+-ATPase activity and increased the intracellular Na^+ concentration and, consequently, cell volume. These effects suggest that IFN-gamma can trigger signaling events that result in the leaky, dysfunctional epithelium that is characteristic of chronic inflammation (4). The potential role of IFN-gamma in IBD diarrhea was supported by a subsequent study of NHE expression in culture and in adult rats. NHE expression was monitored by unidirectional $^{22}Na^+$ influx and by changes in concentration in rat brush-border membrane vesicles; NHE protein and mRNA levels were assessed by Western and Northern blotting. The investigators found that IFN-gamma triggered downregulation of NHE2 and NHE3 expression and activity, which could result in inflammation-associated diarrhea (5).

The Chang team also investigated the intestine's ability to adapt to new sodium absorption requirements following extensive bowel resection. After removing 50% of the proximal rat bowel, investigators found that brush-border hydrolase activity and total cell protein per DNA was comparable to the length of bowel, but basolateral Na^+/K^+-ATPase activity was increased. NHE2 and NHE3 levels increased in the ileum distal to the anastomosis; their expression in the proximal colon increased only after 80% of the bowel had been removed. The investigators concluded that an increase in luminal sodium concentration in the distal bowel following a proximal resection may trigger a compensatory increase in apical NHE gene transcription and protein expression (6).

After several years of work on transmembrane ion movement, Chang and colleagues expanded their efforts to focus on methods of protecting the integrity of the colonic epithelium. Heat shock proteins (HSPs) had been shown to be effective in this regard in animal models of septic shock (7). HSP expression had not been induced in humans because laboratory induction agents are highly toxic. Chang and colleagues discovered, however, that when glutamine is administered to rats with endotoxemia, it reduces mortality dramatically and protects against end-organ damage (7). This protection appears to be associated with a reduction in the release of at least two pro-inflammatory cytokines: TNF-alpha and IL-1 beta (8). Chang and colleagues also observed this in human peripheral blood polymorphonuclear cells (9). Importantly, it is effective when given as sepsis begins, rather than as a pretreatment (7, 8). Thus, glutamine may prove effective in therapy rather than prophylaxis only.

The gut flora may contribute to the protection afforded by glutamine by continuously inducing the expression of HSPs on the surface of colonic enterocytes. By monitoring *E coli* (10) lipopolysaccharide (LPS) and mouse colonic HSP25 levels, the Chang team found that LPS induced HSP25 induction in colonic epithelial cells and may protect the colon from injury by means of filamentous actin stabilization, both under normal and pathophysiological conditions (11). These findings were confirmed in a subsequent article by the Chang team, which reported that HSP expression in rats that had been surgically altered to achieve continuous colonization within the jejunum resulted in improved protection against oxidant-induced transmural stress (12).

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MAYER, LLOYD F, MD

Dr. Mayer received funding from CCFA from 1998 through 2000 to investigate the mechanism of CD8 suppressor T-cell function induced by intestinal epithelial cells.

As early as 1990, a Mayer research team reported an unusually high proportion of T cells with T-cell antigen receptors containing the gene product V-beta 8 in patients with CD (1). They were unable to correlate this finding with the clinical characteristics of the disease and they were not able to connect the RFLP for V-beta 8 with a specific disease. However, they did find evidence suggesting that these T cells were concentrated in diseased bowel tissues (1). They also found that the monoclonal antibody that detects V-beta 8 interacts strongly with an unidentified antigen on epithelial cells and hypothesized that an autoantigen may exist on damaged epithelial cells.

Five years later, Mayer and associates reported that the key to mucosal epithelial cells being able to trigger CD8-positive suppressor T-cell activity depends on an epithelial cell surface non-class I molecule activating a CD8-asociated tyrosine kinase (p561ck); that activation appears to allow the CD8 molecule to bind with the T cell. This linkage appears to be essential for T-cell activation, but not for T-cell proliferation, which suggests that second signal might be necessary for such proliferation. The authors suggest that the second signal might work through the T-cell antigen receptor (2).

CD8-positive suppressor T-cell proliferation may also require a specific epithelial surface structure. Proliferation is blocked by two epithelium-specific monoclonal antibodies--mAB B9 and mAB L12--both of which recognize a 180-kDa glycoprotein (gp180) on the epithelial membrane. gp180 appears to be capable of regulating mucosal immune responses, as it can bind with peripheral blood T cells and activate p56(lck) (3). Expectedly, gp180 is not as plentiful in inflamed intestinal tissue as it is in normal tissue, as indicated by patchy immunohistochemical staining in UC and faint to absent staining in CD. Additionally, gp180 expression is altered and p561ck activity is reduced in IBD tissue (4). Within another 2 years, Mayer and colleagues had determined that the intestinal epithelium triggers CD8-positive suppressor T cell proliferation in conjunction with p56(lck) and the T-cell receptor-associated kinase p59(fyn) (5).

Suppressor T cells are believed to promote oral tolerance in normal tissue (6). The Mayer team tried to determine whether tolerance could be induced in patients with UC or CD by feeding them keyhole limpet hemocyanin (KLH) and attempting to raise anti-KLH antibodies through subcutaneous and booster immunization. KLH-induced T-cell proliferation was reduced in controls but enhanced significantly in patients with CD or UC. Neither oral tolerance nor antibodies to KLH could be raised in these patients. Active immunity may have been triggered in the patients with IBD, indicating a functional defect in the ability of mucosa to suppress an immune response.

Thus, through their methodical exploration of an unknown protein on the surface of the gut epithelium, Mayer and colleagues eventually found several important keys to a crucial component of autoimmune activity in IBD and the development of oral tolerance. Continued exploration of the latter may pave the way to the development of an oral vaccine against antigens responsible for intestinal inflammation.

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Hepatitis C—Destined To Become An Infection Of The Past?

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ABSTRACT

The hepatitis C virus (HCV) currently infects 170 million individuals worldwide, placing them at increased risk for hepatocellular carcinoma, end-stage liver disease, liver transplants, and death. The incidence of hepatitis C has decreased dramatically since it was first identified, primarily as a result of preventive practices. Immunoassays and genotyping techniques now permit an accurate diagnosis and guide selection of therapy. Though viral control methods have improved, however, viral eradication methods have remained only partially effective, allowing the prevalence of infection to remain high. The relatively recent development of in-vitro systems for studying the molecular mechanisms of HCV infection has allowed investigators to identify potential targets for drugs that eradicate the virus or prevent its progression.

INTRODUCTION

Two decades ago, hepatitis C was the most common chronic bloodborne infection in the United States.[1] Since 1989, when the hepatitis C virus (HCV) was discovered, the incidence of this infection has decreased by almost 90%,[2] primarily as a result of preventive practices in blood banking and among high-risk individuals. Unfortunately, the burden of disease has declined much more slowly, leaving more than 3 million persons in the United States and 170 million worldwide, still chronically infected.[2] Many patients became infected during the decade-long lag time between the discovery of a "non-A, non-B" viral agent, and the discovery of its identity—HCV—and its structure. This elevated caseload is expected to persist until virus eradication becomes more effective. Fortunately, community-based prevention programs have been successful in reducing the rate of new infections, and techniques for immunoassays and genotyping can now provide an accurate diagnosis and guide the selection of therapy. The development of growth cultures for studying the molecular mechanisms of HCV infection has improved the potential for identifying targets toward which newly designed virus-eradicating drugs can be directed.

This article presents an overview of the primary issues contributing to the continued prevalence of hepatitis C and explores current treatments and recommendations for improved HCV control.

THE EPIDEMIOLOGY OF HEPATITIS C

HCV: A Brief History

In 1970, researchers discovered an agent other than HAV and HBV in an alarming number of patients with viral hepatitis. This "non-A non-B" hepatitis was initially considered benign, until researchers found that 20% of patients with this infection develop cirrhosis.[4] It took 12 years to identify the causative agent—HCV—and another 7 years to determine its structure. Meanwhile, rates of HCV infection surged, so that by 1988 it became the most common chronic bloodborne infection in the United States, and it remains highly prevalent today.[1,2] Thus far, it has been responsible for approximately 350,000 deaths,[3] and considerable morbidity. It accounts for approximately 60% of all cases of hepatocellular carcinoma, 40% of all cases of end-stage liver disease, and 30% of all liver transplants[5] in industrialized countries. End-stage liver disease and hepatocellular carcinoma from chronic infection are the most common indication for liver transplantation in the United States.

Prevalence

National surveillance programs reveal a detailed picture of the prevalence of hepatitis C and provide insight into the populations most likely to be affected (Table 1), and the individuals at greatest risk (Table 2). Unfortunately, reporting is voluntary and therefore incomplete, so the situation is likely worse than it seems. For every case of hepatitis reported to the Centers for Disease Control and Prevention, as many as 2 to 5 cases are not reported.[2] Consequently, rather than the 3.4 million known cases of HCV infection in the United States, there may be closer to 15 million cases. Given its reported prevalence in other countries (Fig. 1)[3] and current migration trends, the actual prevalence of this disease in the United States could be formidable.

TABLE 1. INCIDENCE OF HEPATITIS C IN VARIOUS AT-RISK CATEGORIES OF U.S. POPULATION.

Intravenous drug use	80%
Multiple sex partners	40%
Homeless	22%
Persons with HIV infection	20%
Incarcerated	15%
Alcohol use	11%-36%
Veterans	8%
General population	1.8%

Key Changes in Demographics

The demographics of hepatitis C have changed considerably over the last 2 decades. Although chronic infection rates remain highest among African Americans, the broad racial disparities of the past have diminished. The simple passage of time and consequent aging of the population have also had a notable effect, with infection rates under age 39 falling dramatically. Individuals with chronic infection today most likely contracted it during the 1980s, when the HCV infection rate was at its peak.[2]

TABLE 2. INDIVIDUALS AT GREATEST RISK FOR HEPATITIS C.

High risk of HCV Infection is associated with:

- Any history of injection drug use
- Contaminated blood or blood products or organ transplantation before July 1990
- Incarceration
- Needlestick or sharp injuries
- Procedures (e.g. injection, vaccination, surgery, transfusion, ceremonial rituals) involving reuse or sharing of contaminated equipment in parts of the world with high HCV prevalence
- Nonsterile contaminated tattooing or body piercing equipment
- Receiving hemodialysis
- Sharing personal items contaminated with blood with an HCV-infected person (e.g., razors, nail clippers, toothbrush)
- Sharing contaminated intranasal cocaine equipment
- Hepatitis B virus infection
- HIV infection
- Children born to mothers with HCV infection
- Undiagnosed liver disease

Moderate risk of HCV Infection is associated with:

- A sexual partner with HCV
- Multiple sexual partners
- Sexually transmitted infection, including HIV and lymphogranuloma venereum
- Traumatic sex that involves the potential for mucosal tearing (sex toys, fisting)
- Vaginal sex during menstruation

Transmission of hepatitis C virus is NOT associated with:

- Coughing
- Food
- Water
- Sharing eating utensils
- Hugging or kissing
- Shaking hands
- Toilet seats
- Other casual contact
- Breastfeeding (unless nipples are cracked and bleeding)
- Oral sex (unless blood exposure is involved)

Figure 1: Estimated prevalence of HCV worldwide, by region.

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Modes of Transmission

In the United States HCV is usually transmitted parenterally – most often through use of injected drugs, especially when needle sharing is involved,[2] and less commonly through occupational exposure among healthcare workers. The next most common exposure is sexual, especially when multiple partners are involved. Transfusion is now only a rare cause because of mandatory blood screening, although many patients with chronic infections were exposed through blood products during the 1970s and 1980s, before blood screening became routine. Perinatal transmission is very uncommon, at least in the United States.[2]

THE VIRUS: A CLOSER LOOK

HCV is an RNA virus of the Hepacivirus genus of the Flaviviridae family that only infects humans. It consists of 6 major genotypes with nucleotide sequences that vary by as much as 30%, and at least 50 subtypes.[6,7] The dominant genotype varies with geographic location and changes over time. Local genotype tracking is crucial for treatment success, since treatment responses vary with genotype. The 3-dimensional structure and active sites of key enzymes involved in the infectious process have been identified; these may serve as targets for drugs that may lead to eradication of the virus and prevent acute infections from becoming chronic.

Natural History of HCV Infection

HCV begins as an acute infection, with HCV RNA levels rising rapidly within 2 weeks of exposure, then leveling off over the following 4 to 6 weeks.[8] Meanwhile, serum aminotransferase levels rise, peaking within 3 months of exposure.[4]

The first line of defense against HCV is the hepatic natural killer (NK) cell, which secretes interferon (INF)-α to inhibit HCV replication.[9] However, HCV can inhibit T-cell proliferation, which facilitates progression to a chronic infection.[4] The role of the anti-HCV antibody is unclear; spontaneous viral clearance has been observed in children with agammaglobulinemia, which suggests that HCV infection can be controlled without elevated levels of anti-HCV antibodies.[4]

Viral clearance occurs in 20% to 40% of patients with acute infection, but the remaining 60-80% develop a chronic infection, which lead to a mild chronic hepatic inflammation. In at least 20% of patients, the resulting necrosis and apoptosis may lead to progressive fibrosis accompanied by nodular regeneration, i.e. cirrhosis, 2-3 decades after the chronic infection begins. Progression to cirrhosis is slow compared with other types of liver disease, but increases with male gender, higher age at exposure, longer duration of infection, immunosuppression, chronic coinfection with hepatitis B or HIV, alcohol use, and obesity. Each year up to 5% of HCV patients with cirrhosis develop liver failure, and up to 4% develop hepatocellular carcinoma.[6]

TESTING FOR HCV

Who Should Be Tested?

The American Association for the Study of Liver Diseases (AASLD) recommends an HCV test for:

a. Anyone who has ever injected an illicit drug - even once;
b. Anyone with an illness associated with hepatitis C, eg, HIV infection or hemophilia, particularly if they received clotting factors before 1987 when viral inactivation procedures were initiated;

c. Those with a history of dialysis, transfusion, or organ transplant before July 1992, when routine blood screening was initiated;
d. Current sex partners of infected individuals, although the risk of transmission in monogamous relationships is very low.[10]
e. Children of infected mothers, preferably at about 18 months of age, although the risk of perinatal transmission is low.

Testing Strategy: An Overview

A routine test for anti-HCV often reveals chronic infection. An HCV RNA test is recommended after a positive anti-HCV test, as well as for patients with unexplained liver disease, immunosuppression, or acute HCV infection. HCV genotyping is recommended if treatment is considered, in order to determine the duration of treatment and to predict the likelihood of a response. A liver biopsy, while not essential, may be considered to assess the extent of liver involvement, to eliminate coexisting conditions, and to determine the prognosis.

Patient Counseling

Infected individuals should receive counseling to prevent them from transmitting the virus. Those who inject or snort illicit drugs should of course be encouraged to stop, or at least to stop sharing or reusing syringes and other drug paraphernalia. All patients should be advised against sharing toothbrushes or shaving equipment; donating body tissues, including blood, organs, or semen;10 drinking alcohol; or taking hepatotoxic medications (acetaminophen may be taken in low doses). They should be encouraged to use condoms, to be vaccinated against hepatitis A and hepatitis B, and to receive yearly flu shots.

Diagnosis of Hepatitis C

The clinical symptoms of HCV infection vary considerably, so the diagnosis is based primarily on laboratory assessments. Since liver enzymes may not be elevated, it is necessary to perform assays specific for Hepatitis C.

Clinical manifestations. During the HCV incubation period (approximately 6-8 weeks after exposure) symptoms may include fatigue, anorexia, malaise, jaundice, a maculopapular rash, mild hepatosplenomegaly, and arthralgia.[6] These symptoms may be only mild, but they worsen with the severity and duration of the infection. Within 2 to 3 decades after exposure, approximately 20% of patients may show signs and symptoms of cirrhosis (ascites, edema, jaundice, easy bruising, variceal bleeding, encephalopathy), but until end-stage liver disease develops, most patients are asymptomatic. Coexisting extra-hepatic disorders – e.g. glomerulonephritis, mixed cryoglobulinemia, porphyria cutanea tarda, vasculitis, depression, and diabetes – are not uncommon in these patients.

Laboratory testing. Screening laboratory tests are used to find anti-HCV. HCV RNA testing should be ordered to document viremia.

Assessment assays. Enzyme immunoassays (EIAs) are used to quantify serum anti-HCV levels, and current third-generation EIAs have high anti-HCV specificity (99%). Antibodies against HCV proteins can be detected by a second-generation recombinant immunoblot assay[4] (RIBA); this increases the specificity of the EIA in low-risk populations, but is rarely necessary for diagnosis or management.

Serum HCV RNA can be quatified by the signal amplification branched DNA assay (bDNA), but is limited by relatively high upper limits of detection for HCV RNA, and lower sensitivity. More sensitive assays are preferred, including PCR-based and transcription-mediated amplification (TMA) techniques with a lower limit of detection of 50 IU/mL or less.[10,11]

Results and their interpretation. Both anti-HCV and HCV RNA are detected in most patients with chronic hepatitis C and later-phase acute hepatitis C. A positive anti-HCV and negative HCV RNA could indicate any of the following: chronic hepatitis C with very low-level viremia, a false-positive EIA, spontaneous or treatment-related HCV clearance, or a false-negative HCV RNA (if the blood is not processed immediately). A positive HCV RNA and negative anti-HCV result may indicate acute hepatitis C, immunosuppression, a false-positive HCV RNA, or a false-negative anti-HCV result. Acute infection can be verified by a positive HCV RNA result obtained 1 week after HCV exposure.[10]

Genotyping. As discussed previously, treatment response varies with HCV genotype.[11] Two genotyping tests are available for use in clinical settings: 1) Direct sequencing (Trugene HCV 5'NC, Visible Genetics, Toronto, Canada); and 2) Reverse hybridization of known

oligonucleotide probes (InnoLiPA HCV II,Innogenetics, Ghent, Belgium).

Liver biopsy. The diagnostic role of liver biopsy in chronic hepatitis C is controversial because of procedure-related risks and the availability of accurate noninvasive tests. Its primary role is to determine the severity of inflammation and the extent of fibrosis, which are used to decide whether treatment is needed.[10] A liver biopsy can also be used to find coexisting liver conditions that cannot be identified by serology, e.g. nonalcoholic fatty liver disease, medication-induced hepatitis, and granulomatous hepatitis.

TREATMENT

The decision to treat a patient with an HCV infection is based primarily on the severity of hepatic involvement, the presence of contraindications, and the patient's age, co-morbid conditions, and willingness to be treated. Genotype is also considered, because patients with genotype 2 or 3 have high response rates.

Treatment of Chronic Hepatitis C

The overall goal is a sustained virologic response (SVR), ie, the inability to detect serum HCV RNA with a sensitive assay 6 months following the end of treatment.

Standard treatment for chronic HCV infection is pegylated (covalently bonded to polyethlene glycol) Interferon and ribavirin. Interferon is usually given as IFN alfa-2a 180 μg/wk, regardless of body weight, or IFN alfa-2b 1.5 μg/kg weekly, and the dosage of ribavirin varies with weight and genotype, which also influences the duration of treatment.

Patients with Genotype 1

Genotype 1 is the most common genotype in the U.S., accounting for approximately 70% of infections. Treatment is not recommended for genotype 1 patients with mild lesions, except young adults before their childbearing years and persons in professions that put them at risk to transmit the virus (e.g., surgeons, nurses, dentists).

A 48-week course of pegylated IFN is recommended, along with high-dose ribavirin (1000-1200 mg/d based on weight; maximum 1600 mg/d).[11] HCV RNA should be evaluated at Week 12 and compared with baseline to identify patients who are not likely to achieve an SVR. If no change in HCV RNA is observed, treatment should be discontinued. If the HCV RNA level is still detectable at Week 12 but has decreased 102-fold from baseline, treatment should continue until Week 24. If HCV RNA is still detected at Week 24, treatment should be discontinued, because an SVR will not be achieved. In patients with undetectable virus at Week 12 or a 10^2-fold decrease in viral load at Week 12 and undetectable virus at Week 24, therapy should be continued until Week 48. Data are emerging, however, to suggest that patients with a 102-fold drop who do not achieve viral negativity at week 12 may improve the chance of an SVR with a 72-week regimen. On the other hand, patients who achieve a rapid viral response (defined as viral negative at 4 weeks) may only need 24 weeks of treatment.

These regimens give patients a 40% to 50% chance of achieving an SVR[11] and a >98% chance of long-term remission – which many hepatologists consider a cure.

Patients with Genotype 2 or 3

Most of the remaining patients have genotypes 2 or 3, which have high response rates and should be treated regardless of the severity of the liver disease. A 24-week regimen consists of pegylated IFN accompanied by low-dose ribavirin (800 mg/d, regardless of weight). HCV RNA levels are usually undetectable after Week 4; HCV RNA should be assessed 24 weeks after therapy is discontinued to determine whether an SVR has been achieved. With this regimen these patients have a 70% to 85% chance of achieving an SVR.

Patients with Genotypes 4, 5, or 6

These cases are uncommon, so limited data exist regarding the likelihood of an SVR. The current recommendation is for the same regimen as genotype 1, with assessments of virologic response at weeks 48 and 72.

Treatment of Acute Hepatitis C

When an acute infection is suspected, the physician must decide whether and when to treat the patient. The general recommendation is to wait 2 to 3 months to treat, because: 1) acute infections often resolve on their own (viral clearance can occur within 3 months of exposure); and 2) disease progression rates are low immediately after exposure. Once the decision to treat is made, the physician is faced with a dearth of firm guidelines for choosing the best treatment. In one small study, INF alfa-2b 5 MU daily for 4 weeks followed by

TABLE 3. GUIDELINES FOR HEPATITIS C VIRUS RNA TESTING.

Clinical situation	Test to use	Interpretation and comments
Acute infection suspected	Qualitative PCR or real-time PCR	Check HCV RNA and HCV antibody 4-6 wk after exposure Check HCV RNA at 8-12 wk; if positive, consider therapy Check HCV RNA and HCV antibody 4-6 mo after exposure
Chronic infection suspected† HCV antibody positive	Qualitative PCR or real-time PCR	HCV RNA positive: patient is chronically infected HCV RNA negative: patient is most likely not infected, but low-level or intermittent viremia possible. Repeat RNA testing recommended in 6-12 mo
HCV antibody negative but unexplained liver disease or immunocompromised	Qualitative PCR or real-time PCR	HCV RNA positive: patient is chronically infected, unless acute HCV infection is supported by clinical situation. HCV RNA negative: patient is most likely not infected, but low-level or intermittent viremia possible. Repeat RNA testing recommended in 6-12 mo
HCV antibody and RNA positive, eligible for treatment	Quantitative tests such as quantitative PCR, bDNA, or real-time PCR	>800 000 IU/mL is considered high, more difficult to treat Use same quantitative assay before treatment and measure 4- and 12-wk responses
Infant born to HCV positive mother; infant still antibody positive at 18 mos	Qualitative PCR or real-time PCR	HCV RNA positive: patient is chronically infected HCV RNA negative: patient is most likely not infected, but low-level or intermittent viremia possible. Repeat RNA testing recommended in 6-12 mo

Abbreviations: bDNA, branched-chain DNA; HCV, hepatitis C virus; PCR, polymerase chain reaclion.
†Most recent exposure to HCV more than 6 months prior.
(Scott JD, Gretch DR. Molecular diagnostics of hepatitis C virus infection: a systematic review. JAMA. 2007;297:724-732. Copyright © 2007, American Medical Association. All rights reserved.)

5 MU 3 times weekly for 20 weeks resulted in an SVR rate of 98%.[4] Pegylated INF alfa-2b 1.5 μg/kg weekly for 12 weeks resulted in SVR rates of 95%, 92%, and 76%, depending on whether INF therapy was initiated at Week 8, 12, or 20, respectively.[12] Guidelines for HCV RNA testing appear in Table 3.[13]

TREATMENT-RELATED ADVERSE EFFECTS

The adverse effects (AEs) of IFN and ribavirin are responsible for 10% to 20% of patients withdrawing from therapy and 20% to 30% of patients requiring a reduced dose. IFN is associated with bone marrow depression, neuropsychiatric effects, autoimmune disorders, and flu-like symptoms; ribavirin is associated mainly with hemolytic anemia and rash (Table 4).[14] Each AE is managed as it would be as a primary condition. Autoimmune disorders (especially thyroiditis) may continue after therapy has ended. Their development warrants immediate discontinuation of therapy. In patients with hemolytic anemia, ribavirin dosing often has to be reduced dose or stopped. These patients may respond to erythropoietin, but this drug is expensive and not universally reimbursed. Fertile men and women must use 2 methods of birth control during treatment, because ribavirin is teratogenic. Neuropsychiatric side effects (depression, anxiety, and agitation) are often the most difficult effects to manage and have been associated with relapse to alcohol and drug use.

FUTURE DEVELOPMENTS

HCV polymerase has emerged as a key target for drug development. Three types of inhibitors have been identified: nucleoside analogs (which prevent polymerase elongation), nonnucleoside analogs (which disrupt the initiation of polymerization),[15] and pyrophosphate mimics (which block the polymerase active site).[16] The in vitro efficacy of the nucleoside inhibitors (e.g. valopicitabine) and nonnucleoside inhibitors (e.g., benzothiazidines) appears to be influenced by genotype.[17] Although the nucleoside valopicitabine, NM283 (Idenix) has already demonstrated anti-HCV activity in phase 1 and phase 2 trials, further development of this drug has been halted because of gastro-intestinal toxicity. Additional trials are currently in development[17] using the nucleoside R1626 which showed significant antiviral activity in a phase IIa study.[18]

TABLE 4. ADVERSE EFFECTS OF HEPATITIS C THERAPIES INTERFERON.

Common (≥10%)

- Mild bone marrow suppression (anemia, leukopenia, thrombocytopenia)
- Depression
- Insomnia
- Fatigue and irritability
- Weight loss, anorexia
- Fever, myalgia, headaches and flu-like symptoms
- Alopecia
- Skin irritation at injection site
- Nausea, vomiting and diarrhea

Occasional (2%-9%)

- Retinopathy (usually not clinically significant)
- Exacerbation of autoimmune condition (e.g., autoimmune hepatitis, autoimmune thyroiditis, rheumatoid arthritis, psoriasis)
- Congestive heart failure and arrhythmias

Rare (≤1%)

- Severe bone marrow suppression
- Seizures
- Tinnitus and hearing loss
- Hyperglycemia
- Renal failure
- Pneumonitis

Ribavirin

Common (≥10%)

- Hemolytic anemia (dose dependent)
- Fatigue
- Rash and pruritis
- Nasal stuffiness
- Cough

Small interfering RNAs and antisense oligonucleotides are also being investigated for their efficacy against HCV. These may require delivery directly into the liver with molecular vehicles currently under investigation. These include lentiviral vectors, which allow their "passengers" to be integrated into the host genome, and vectors from nonhuman sources.[19]

Protease inhibitors (PIs) are the most promising new therapeutic agents. Since early clinical trials revealed that viral mutants emerge after short courses of PIs alone, current phase 2 and phase 3 trials use them with INF, plus or minus ribavirin. In the phase II trials (PROVE 1 and PROVE 2) presented at the 2007 meeting of the AASLD, telaprevir in combination with interferon and ribavirin achieved higher rates of rapid viral response and a lower risk of relapse (which suggests higher rates of SVR) even with shorter treatment lengths.[20,21]

Buceprevir is another oral protease inhibitor that has completed phase II trials and is heading into phase III trials. This drug is well tolerated and displays significant antiviral activity.[22] Results of the phase II trial were presented at Digestive Diseases Week in May 2008.

SUMMARY

Over the last 2 decades, significant progress has been made in the control of hepatitis C. Despite the lack of an anti-HCV vaccine, the incidence of this disease has decreased, though its prevalence remains high. Recent investigations into its molecular nature have already revealed potential targets for antiviral pharmaco-therapeutic strategies, and may someday eliminate hepatitis C as a primary cause of liver disease.

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