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Contents

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■ BIOTECHNOLOGY RESOURCES

Cancer Biomedical Informatics Grid (caBIG): Accelerating Cancer Research Through Shared Data and Biomedical Research Tools

The advances in cancer research methods and technologies in recent years have resulted in an explosion of information and knowledge about all forms of cancer and cancer treatment. Information from diverse areas of research such as genomics, proteomics, clinical trials, and many other fields is rapidly providing us with new insights and answers. With these advances, the richness of information available within the cancer research community, and the growing interest in transdisciplinary team science, enormous opportunities have arisen to improve our ability to eliminate suffering and death due to cancer. However, most cancer researchers currently make use of only a small fraction of the existing data and have no comprehensive way of providing others easy access to the data that they, themselves, have collected. Without a unifying infrastructure or common set of standards for biomedical research information, researchers cannot easily share technologies or benefit from the innovative informatics tools developed by others.

To address these challenges, the cancer Biomedical Informatics Grid (caBIG) initiative was announced in July 2003, under the coordinating supervision of the NCI. caBIG aims to create a voluntary virtual network (or grid) that links researchers, institutions, and organizations together in a manner that promises to accelerate progress in all aspects of cancer research—from etiologic research to prevention, early detection, and treatment. caBIG will promote "bench-to-bench," "bench-to-bedside," and "bedside-to-bench" initiatives. With the current lack of interoperable standards, this is not yet possible. CCR scientists are participating in the caBIG initiative and working on software to enable the integration of clinical and genomic data, thereby allowing the entire caBIG

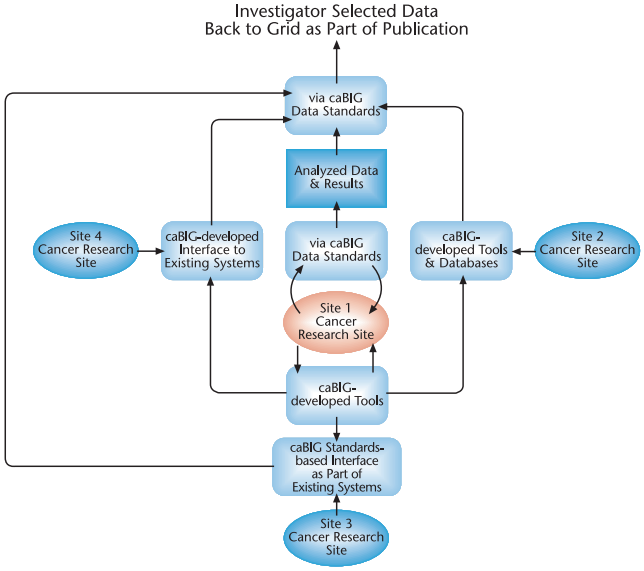

Figure 1. Schematic representation of caBIG. Federated caBIG access enables a researcher at any caBIG cancer research site (in this example, Site 1) to perform a single query search of all caBIG information via caBIG-developed tools. The researcher at Site 1 can then analyze data and produce results. The researcher can then choose to enter his/her data into caBIG via caBIG data standards. Other cancer research sites, via caBIG-developed tools, databases, or various interfaces, can then access these results.

community to share in their experience and data.

caBIG will address the need for comprehensive and easily understood tools for clinical data management; modular, interoperable biomedical informatics software; and novel statistical methods. caBIG will also provide access to data in standard formats and ultimately offer training, support, and related documentation for researchers. This will enable caBIG to offer the structure and resources to facilitate the connection between geographically dispersed groups, as well as individual researchers working in a variety of scientific areas (Figure 1). By providing its products as open source, in an open development environment, caBIG is an open-access, voluntary initiative, and anyone in the biomedical research arena can participate in or contribute to caBIG activities.

To date, 50 NCI-designated cancer centers and the CCR have contributed to the development of the vision, approach, and structure of the pilot phase of caBIG, based on common community needs and concerns. Three specific areas known as Workspaces were identified to begin developing biomedical research tools: Integrative Cancer Research (ICR), Tissue Banks and Pathology Tools (TBPT), and Clinical Trial Management Systems (CTMS). Two crosscutting Workspaces were also established: Vocabulary and Common Data Elements (VCDE) and Architecture, both of which were established to support consistency and common standards across the caBIG community. Three strategic-level Working Groups (Strategic Planning, Training, and Data Sharing and Intellectual Capital) were established to provide guidance on issues of common concern and scalability to the caBIG community as a whole.

The caBIG ICR Workspace will produce modular and interoperable tools and interfaces that provide for integration between biomedical informatics applications and data. This will ultimately enable translational and integrative research by providing for the integration of clinical and basic research data. Next year, systems will be developed that offer grid-enabled access to a variety of microarray data sources available to the cancer center community. These systems will follow caBIG-standardized access and retrieval mechanisms and will provide for federated access to the data when compatible microarray browsing and analysis tools are used. Several centers, as well as researchers from the CCR, have offered access to large amounts of microarray data as part of the pilot, and other centers have offered tools and analytical methodologies on which to test and validate them. Other early products of the Workspace include grid-enabled access to pathway, proteomic and genomic data, and analytical tools.

The caBIG TBPT Workspace is providing for the integration, development, and implementation of tissue and pathology tools. The full suite will provide for unprecedented abilities to manage, query, retrieve, and harness tissue specimen data and annotations across a federated infrastructure. This Workspace has targeted the release of caTISSUE, a modular, open-source specimen inventory and tracking system that will encompass a core database module for those centers in need of new solutions, as well as application programming interfaces (APIs), software development toolkits (SDKs), and additional annotation modules for those centers with legacy systems that wish to link into the virtual tissue repositories and query across cancer centers.

The caBIG CTMS Workspace will develop a comprehensive set of modular, interoperable, and standards-based tools. This Workspace has identified several high-priority modules including adverse events, laboratory interfaces, a regulatory reporting interface, and financial/billing. As a near-term deliverable, the Workspace will develop a complete solution for a cancer center currently in need of a primary adverse-events data-management system. This tool will be an open-source adverse-event platform that provides a customizable data collection system that complements existing systems by providing reporting, integration, and transformation capabilities, resulting in an enhanced ability to collect, share, and analyze data across organizations.

The VCDE and caBIG Architecture Workspaces are tasked with ensuring that all the tools created by other Workspaces are interoperable and use common standards and unifying architecture. Collectively, their primary focus is the formulation of caBIG standards for common vocabularies, common data elements, ontologies, and data to guide other caBIG participants, as well as collaborating with other caBIG groups to ensure the formation of a unifying architecture for caBIG based on recognized architectural standards.

A list of currently available standards and tools being used to support caBIG activities is available on the caBIG web site at http://caBIG.nci.nih.gov/inventory. This list will continue to grow as caBIG evolves and project activities progress. caBIG is open to anyone who wants to access it and contribute. For additional information on the activities of these Workspaces and Working Groups, and on the caBIG initiative in general, go to http://caBIG.nci.nih.gov. Please address questions to caBIGinfo@cancer.gov.

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Reporting High-Impact Manuscripts

High-impact manuscripts should be reported to Tracy Thompson (thompstr @mail.nih.gov), Chief, CCR Office of Communication, as soon as possible **after acceptance but before publication.** Please include the publication date, an electronic or hard copy of the manuscript, and the journal name. High-impact manuscripts include but **are not limited to** papers that reflect a significant advance in your field or papers in any of the following areas: public health; tobacco-related issues; new technological advances; imaging; obesity, dietary fat, energy balance; nanotechnology; molecular targets; stem cells; angiogenesis; or combination therapies.

DNA Methylation Is Associated with Histone Lysine Methylation in Mice

Yan Q, Huang J, Fan T, Zhu H, and Muegge K. Lsh, a modulator of CpG methylation, is crucial for normal histone methylation. *EMBO J* 22: 5154-62, 2003.

DNA methylation and covalent histone modifications are important epigenetic characteristics of mammalian chromatin. Euchromatin, an "open" form of chromatin, usually contains single-copy genes, shows low levels of DNA methylation, and shows high methylation levels at lysine 4 of histone 3 (H3-K4). On the other hand, heterochromatin, a repressed form of chromatin, is associated with silenced repetitive elements, high levels of DNA methylation, and high methylation levels at lysine 9 of histone 3 (H3-K9); the latter serves as a recruitment site for the major heterochromatin-binding protein HP1.

Much of our knowledge about epigenetic modifications in chromatin has been gained from lower organisms. For example, in fission yeast, H3-K9 methylation and binding of the HP1 homolog are crucial for heterochromatin formation and gene silencing (Nakayama J et al. *Cell* 101: 307-17, 2000; Nakayama J et al. *Science* 292: 110-3, 2001). However, fission yeast lacks DNA methylation and therefore cannot reveal the interplay of these distinct epigenetic modifications. In *Neurospora* and *Arabi-dopsis*, DNA methylation depends on H3-K9 methylation (Tamaru H and Selker EU. *Nature* 414: 277-83, 2001; Jackson JP et al. *Nature* 416: 556-60, 2002). The preferred methylation site in lower organisms is a CNG motif, whereas in mammals the dominant methylation site occurs at the CG motif.

To understand the relationship and interplay of different epigenetic modifications in mammals, we examined the association between DNA methylation and histone methylation in Lsh$^{-/-}$ mice. Lsh, a member of the SNF2 family of chromatin remodeling proteins, is crucial for normal embryogenesis. Lsh$^{-/-}$ mice are smaller than Lsh$^{+/+}$ mice and die within a few hours after birth with signs of renal and lymphoid defects. Lsh is a component of pericentric heterochromatin, and loss of Lsh results in a greatly perturbed heterochromatin organization with reduced DNA methylation at pericentric sequences and other repetitive elements. We investigated embryonic tissue and cell lines derived from Lsh$^{-/-}$ mice for alterations of heterochromatin organization, particularly histone methylation.

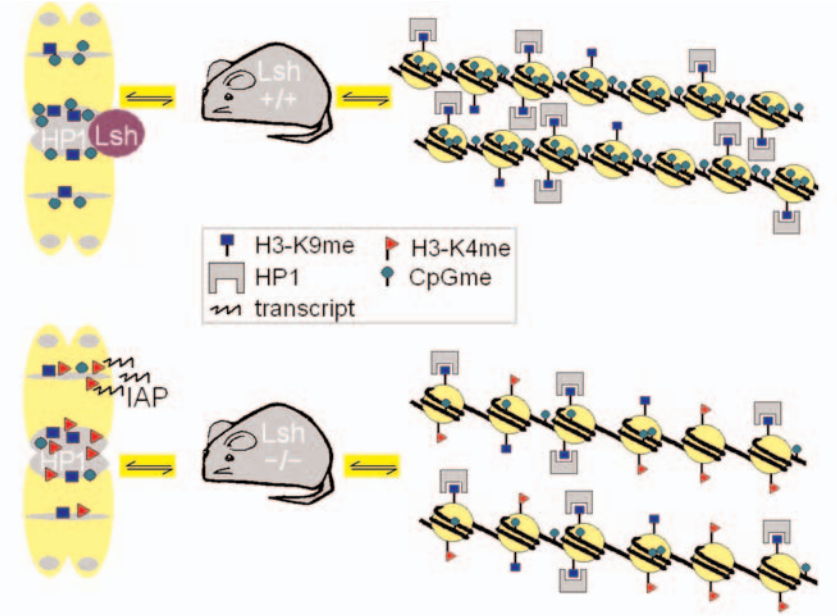

Figure 1. Model of heterochromatin formation in Lsh$^{+/+}$ (*top*) and Lsh$^{-/-}$ (*bottom*) mouse cells. *Left:* Chromosomal level. The gray areas depict heterochromatin, including pericentric and telomeric regions. *Right:* Nucleosomal organization with methylation and HP1 (a heterochromatin-binding protein) binding patterns. Compared with Lsh$^{+/+}$ mice, Lsh$^{-/-}$ mice show the same level of H3-K9 methylation (H3-K9me), a lower level of CpG methylation (CpGme), and perturbed heterochromatin organization. In addition, Lsh$^{-/-}$ mice show increased H3-K4 methylation (H3-K4me) and transcriptional reactivation of retroviral intracisternal A-particle (IAP) elements, neither of which is seen in Lsh$^{+/+}$ mice.

We found that HP1 binding and H3-K9 methylation, hallmarks of heterochromatin, are not altered in the absence of Lsh, despite the loss of CpG methylation (Figure 1). In contrast, H3-K4 methylation, a euchromatic marker, was increased at heterochromatic sites such as pericentric regions or at repetitive elements. Associated with the increased H3-K4 methylation was transcriptional reactivation of normally silenced retroviral intracisternal A-particle (IAP) elements.

These findings led us to three conclusions. First, H3-K4 and H3-K9 methylation can be independently regulated in mice. Although both modifications are mutually exclusive *in vitro* on one histone 3 tail, *in vivo*, distinct nucleosomes are modified and independently regulated. In contrast, mutations of the *Arabidopsis* Lsh homolog result in decreased H3-K9 methylation and increased H3-K4 methylation (Gendrel AV et al. *Science* 297: 1871-3, 2002), demonstrating species-dependent differences in chromatin formation.

Second, we did not observe that CpG methylation directly controlled H3-K9 methylation or HP1 binding, although these heterochromatic modifications

are frequently associated. Instead, CpG methylation was inversely related to H3-K4 methylation. The demethylating drug azacytidine mimicked the effects of Lsh deletion (without depleting Lsh from heterochromatin), which suggests that CpG hypomethylation contributes to the maintenance of heterochromatin structure by preventing the establishment of euchromatic markers.

Third, transcriptional reactivation at the retroviral IAP element did not correlate with H3-K9 methylation or HP1 binding level but with increased H3-K4 methylation levels. This observation highlights the importance of H3-K4 methylation for transcriptional control in mice.

In brief, Lsh regulates two important epigenetic modifications in mice: DNA methylation and histone methylation. The inverse relationship between CpG methylation and H3-K4 methylation is crucial for transcriptional control of repeat elements usually embedded in heterochromatin. Lsh appears to operate as a guardian of normal heterochromatin structure and function, ensuring silencing of "parasitic" elements in the mammalian genome. Understanding such basic mechanisms underlying distinct epigenetic modifications should provide insights into a number of pathologic processes—such as cancer, inherited Rett syndrome, and ICF syndrome—that depend on abnormal chromatin structure.

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■ TRANSLATIONAL RESEARCH

Insulin-like Growth Factor I Promotes Multiple Myeloma Cell Migration and Invasion

Qiang Y-W, Yao L, Tosato G, and Rudikoff S. Insulin-like growth factor I induces migration and invasion of human multiple myeloma cells. *Blood* 103: 301-8, 2004.

Multiple myeloma is an incurable form of lymphoid cancer associated with accumulation of malignant plasma cells (end-stage B-lineage lymphocytes) in the bone marrow. Although the disease constitutes only a small percentage of lymphoid cancers, it is responsible for approximately 20% of lymphoid cancer–related deaths because of its nearly 100% mortality rate.

The molecular lesions contributing to the initiation and progression of multiple myeloma are poorly understood. However, it seems clear that the malignant plasma cells associated with the disease respond to a number of growth factors, such as insulin-like growth factor I (IGF-I) and interleukin 6 (IL-6), both of which appear to contribute to survival and proliferation. One aspect of myeloma biology that has received very little attention is the mechanisms regulating the motility and invasion of the malignant cells. In the initial stage of the disease, circulating myeloma plasma cells home specifically to bone marrow. These cells must first bind to the surface of the blood vessel endothelium and then penetrate the endothelial membrane to reach the bone marrow compartment. Once in the bone marrow, the malignant cells must interact with, and likely move through, bone marrow stromal cells from primary to secondary sites. As IGF-I has been shown to act as a chemotactic factor for several other cell types, we have recently examined the ability of this molecule to promote myeloma cell migration and invasion.

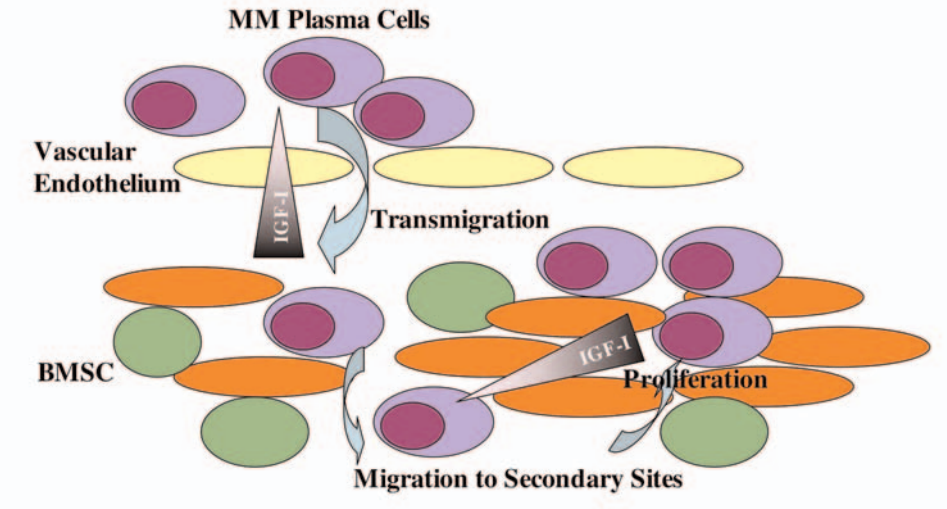

Figure 1. Model for the role of insulin-like growth factor I (IGF-I) gradients in regulating the migration and proliferation of multiple myeloma (MM) plasma cells. IGF-I produced by certain bone marrow stromal cells (BMSC) may form a gradient that, at distal, lower concentrations (such as in blood vessels) promotes migration to areas of higher concentration where thresholds are reached that induce proliferation. Local gradients within the bone marrow may also promote migration to secondary sites.

The ability of IGF-I to induce migration through vascular endothelial and bone marrow stromal cells was assessed by using a transwell assay in which polycarbonate membranes were pre-coated with either normal human vascular

endothelial cells or two phenotypically different bone marrow stromal cell lines. IGF-I induced migration of malignant plasma cells through all three cell types, with a bell-shaped, dose-dependent response curve, classic for chemotactic factors wherein migration is promoted at low concentrations, but inhibited at higher concentrations. Interestingly, IGF-I–mediated proliferation increased with concentration, attaining maximum levels at the same concentrations that inhibited migration. Proliferation plateaued at these concentrations but did not decrease.

Previous studies of IGF-I–induced proliferation (Qiang Y-W et al. *Blood* 99: 4138-46, 2002) revealed activation of two intracellular signaling pathways—the MAPK and PI-3K cascades. Use of inhibitors targeting each of these pathways revealed that both proliferative and anti-apoptotic effects were associated with the PI-3K pathway; inhibition of MAPK had no effect on either biologic property. The PI-3K downstream element Akt was found to be critical in regulating both proliferation and anti-apoptosis. Migration has generally not been associated with the Akt branch of the PI-3K pathway; therefore, an analysis was conducted of other protein families associated with migration in other cell types. These studies revealed activation of RhoA, a member of the family of small GTPases regulating stress fiber formation, and PKCμ, a member of the protein kinase C family, various isotypes of which have been shown to play important roles in cytoskeletal reorganization and motility. Inhibition of PI-3K blocked activation of RhoA and PKCμ indicating that both are downstream of PI-3K.

To associate biologic phenomena with biochemical pathways, a series of proliferation and migration experiments were performed in the presence of a number of specific pathway inhibitors. Results were as follows: 1) PI-3K inhibitors blocked both proliferation and migration. 2) An Akt inhibitor prevented proliferation, but not migration. 3) RhoA and PKC inhibitors blocked migration but had no effect on proliferation. 4) MAPK inhibitors had no effect on proliferation or migration. These studies show that both proliferation and migration are mediated through the PI-3K pathway with PI-3K/Akt regulating proliferation and PI-3K/RhoA, PI-3K/PKC regulating migration. Additionally, activation of both RhoA and PKCμ (as well as possibly other PKC family members) is required for migration, as inhibition of either prevents migration.

The observation that myeloma cells migrate at low concentrations of IGF-I, but proliferate at higher concentrations at which migration is inhibited, raises an interesting question: How does a cell determine which path to follow? This has led us to propose a model (Figure 1) wherein local gradients of IGF-I produced in the bone marrow regulate both processes. The characterization of IGF-I as a promoter of both proliferation and migration/invasion of myeloma cells has given further impetus to the development of IGF-I/IGF-I–receptor antagonists as possible therapeutic agents in the treatment of both multiple myeloma and other cancers.

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FROM THE DIRECTOR'S OFFICE

Patient Care and the Ethics of Clinical Research

The overarching goal of clinical research is to develop knowledge that can be generalized to improve health and our understanding of human disease. The pursuit of this goal should always be guided by basic ethical principles. Eight requirements must be fulfilled to make clinical research ethical (Figure 1).

First, the research must reflect a collaborative partnership between the researchers and the community from which study participants are selected. Typically, a collaborative partnership is manifested through support from the public for the research's funding, the participation of advocacy groups, and inclusion of lay or patient representatives in research advisory and oversight boards such as institutional review boards (IRBs). Second, the research must be socially valuable, addressing meaningful gaps in therapy or scientific understanding.

Third, for clinical research to be ethical, the study methods must be able to produce reliable and valid data, so that there is a reasonable chance that the data will be able to advance therapy or contribute knowledge. Trivial questions, invalid or biased methods, and poor statistical techniques are unethical because flawed science cannot justify risk or inconvenience to research participants. Fourth, subject selection must be fair. The eligibility requirements and recruitment strategy must be defined by the scientific objectives of the research study, not by social vulnerability or preference.

Fifth, the research must have a favorable risk-benefit ratio. Although risks can rarely be eliminated, they should be minimized. Similarly, the potential benefits to the participants and to future patients should be enhanced.

The sixth ethical requirement for clinical research is that it must also undergo

Figure 1. Requirements of ethical research (in brief). Adapted from Emanuel EJ et al. *JAMA* 283: 2701-11, 2000.

independent review by a committee of peers and laypersons, such as an IRB. Such review is intended to provide unbiased evaluation of the scientific and ethical aspects of research, as well as institutional and public accountability for that research. Seventh, subjects must offer their informed consent to participate in research.

The eighth requirement, which is not commonly emphasized, is that respect for research participants must be maintained. Researchers must respect the participants' rights to privacy and recognize the continued voluntary nature of study participation. However, ethical requirements for clinical research do not end when individuals enter into a research study. Individuals must be treated with respect from the time they are approached—even if they refuse enrollment—throughout their participation and even after their participation ends.

An essential component of respect for participants is maintaining their privacy and protecting their confidentiality. This is a necessary component, not because of HIPPA or other legal mandates, but because of the basic underlying ethical principles of clinical research. Frequently, greater emphasis is placed on fulfilling the other ethical requirements of clinical research; respecting patients after their enrollment in a study can be easily neglected.

Typically, patients with cancer who are evaluated at a clinical research center will have traveled appreciable distances from their homes and loved ones to participate in studies. Mortality issues are ever present in their minds along with deep concerns and fears about their health and future. They come to a clinical research center for hope and help in dealing with these life and death issues. Within the center, much or all of their care occurs in the outpatient clinic. There, due to clinic design, limitations of space, and limitations regarding caregivers' time, interactions between professional staff and patients may well occur in the waiting room or other public areas. Physicians often gather in hallways to present and discuss cases, and patient check-in and check-out areas are often one and the same, such that patients exiting, carrying all the emotion of the moment, are directed back to the waiting room to bear their burdens in public. Heavy burdens are common for patients with cancer, and they should be allowed time and space to deal with their complex issues privately and not be forced to deal with them publicly. Risks exist for patient confidentiality and dignity to be breached from clinic sign-in to sign-out. Crowded conditions complicate these issues and potential solutions. Crowding also limits appointments for new patients, and the inability to effectively bring new patients into protocols prolongs time to study completion, and so introduces additional ethical considerations.

If we are to fulfill the eighth requirement for ethical research, we must respect patients as persons by protecting their privacy and dignity during the delivery of care. One model of outpatient care that seeks to do this successfully involves physical and functional compartmentalization of care delivery. In this model, waiting rooms are designed from a patient perspective and are dedicated in their use. Hallways are only for walking and examination rooms are only for examinations. Patient consultation rooms exist in sufficient numbers to accommodate the prolonged presentation and discussion of protocols by multiple investigational team members and caregivers—physicians, research nurses, nurse practitioners, social workers—and the list can be expanded to consultation for such issues as self-image, spiritual support, and psychiatry. Finally, caregiver-only areas exist for multidisciplinary planning, along with physician dictation areas and radiology review areas.

By making waiting, examination, patient-consultation, and caregiver areas physically distinct, with directed patient flow, confidentiality and respect for persons can be maintained, and the efficiency of clinic operation can be enhanced. Emphasizing clinic structure and function as being patient focused contributes to our mission and to meeting the requirement for respecting participants' privacy in conducting ethical clinical research.

■ **Manish Agrawal, MD**
■ **Ronald Gress, MD**

A Safer Smallpox Vaccine for Immunocompromised Persons

Edghill-Smith Y, Venzon D, Karpova T, McNally J, Nacsa J, Tsai WP, Tryniszewska E, Moniuszko M, Manischewitz J, King LR, Snodgrass SJ, Parrish J, Markham P, Sowers M, Martin D, Lewis MG, Berzofsky JA, Belyakov IM, Moss B, Tartaglia J, Bray M, Hirsch V, Golding H, and Franchini G. Modeling a safer smallpox vaccination regimen, for human immunodeficiency virus type 1-infected patients, in immunocompromised macaques. *J Infect Dis* 188: 1181-91, 2003.

The possible consequences of the deliberate release of smallpox by bioterrorists have raised concern about our ability to vaccinate all persons who might be at risk of infection. The only currently available smallpox vaccine, Dryvax (Wyeth Laboratories, Marietta, PA), is a preparation of live vaccinia virus that was used in the United States until routine vaccination was halted in 1972.

Dryvax may cause adverse effects when injected in persons with immunodeficiency disorders. Because the number of people with weakened immune systems has increased worldwide (as a result of aggressive chemotherapy regimens in cancer patients, the HIV-1 epidemic, and an increase in the number of organ transplants), the risks associated with Dryvax vaccination may affect a larger portion of the population than before. In some immunocompromised persons, Dryvax may produce serious or even fatal complications, including "progressive vaccinia," in which the initial vaccination site continues to enlarge and virus spreads to other sites on the body. In the past, most cases of progressive vaccinia occurred in infants with congenital immune defects or in adults with acquired immunodeficiency disorders (usually resulting from leukemia or lymphoma), but a few cases were also observed in persons severely immunocompromised from HIV infection.

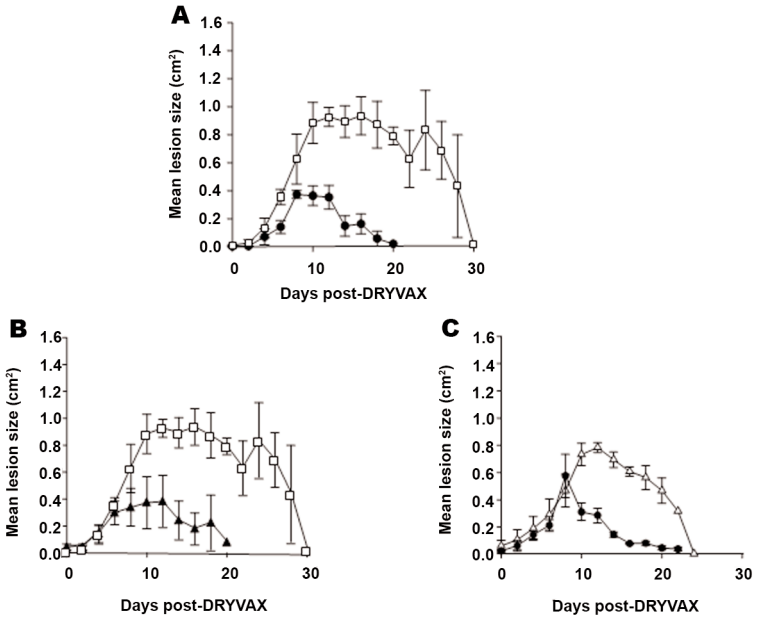

Figure 1. Mean lesion size (and standard deviation) over time in rhesus macaques. *A:* Healthy macaques plus Dryvax (●) and immunodeficient macaques plus Dryvax (❑). *B:* Immunodeficient macaques plus Dryvax (❑) and macaques immunized with the highly attenuated strain of vaccinia NYVAC before simian immunodeficiency virus (SIV) infection plus Dryvax (▲). *C:* Macaques immunized with NYVAC before SIV infection plus Dryvax (●) and after SIV infection plus Dryvax (△).

One approach to vaccine safety has been the development of attenuated vaccine

Continued on page 8…

ADMINISTRATIVE LINKS

2005 NCI Combined Intramural PI Retreat

Don't wait until it is too late! September is here, and it is time to start registering for the 2005 NCI Combined Intramural Principal Investigator Retreat, which will be held January 12 and 13, 2005, at the Bethesda North Hotel and Conference Center across from the White Flint Metro Station. You may register online at http://www.palladianpartners.com/retreat2005, starting September 3, 2004. All poster abstracts must also be submitted online via the registration web site. Poster abstract submission will close as of October 8, 2004. If you have any questions, please contact CCR's coordinators, Jennifer Kwok (kwokj@mail.nih.gov), Julie Hartman (jf211u@nih.gov), or Barbara McElroy (SAIC, mcelroy@mail.ncifcrf.gov).

NCI Administrative Intranet: Travel Toolbox

If you need additional information regarding the NIH travel policy and procedures, try the new **Travel Toolbox** link, located on the NCI Administrative Intranet at http://camp.nci.nih.gov/admin/travel.html.

The Travel Toolbox provides access to policies, procedures, delegations, forms, contacts, and reference information. It also includes the popular "travel topic paks," which are packed with helpful information and links relating to both local and foreign travel.

...continued from page 7

viruses incapable of spreading from the inoculation site. In our study, we tested the hypothesis that immunization of an immunocompromised person with a highly attenuated poxvirus would prevent adverse consequences from subsequent Dryvax vaccination. In addition, we chose to maintain Dryvax in a prime-boost approach because Dryvax is the only known vaccine proven to protect against smallpox. We first studied the effects of Dryvax in rhesus macaques that had $CD4^+$ T-cell depletion induced by infection with simian immunodeficiency virus (SIV) or simian/human immunodeficiency virus (SHIV). We found that Dryvax produced significantly larger skin lesions in immunocompromised than healthy macaques and that the lesions took significantly longer to heal (Figure 1A).

To assess whether we could prevent the development of these enlarged Dryvax lesions, we tested the effect of prior immunization with the highly attenuated strain of vaccinia virus NYVAC, in which a deletion of 18 open reading frames has been generated. We found that when macaques were immunized with NYVAC before infection with SIV and then immunized with Dryvax, the size of Dryvax lesions was significantly reduced and took less time to heal compared with the lesions of immunocompromised macaques immunized with Dryvax only (Figure 1B). If NYVAC immunization was delayed until after SIV infection and Dryvax vaccination, when the animals had already developed $CD4^+$ T-cell depletion, a lesser degree of protection was observed (Figure 1C). However, protection could be somewhat improved by shortening the interval between NYVAC and Dryvax vaccination. Thus, in the context of established immunodeficiency, protection against smallpox by means of a prime-boost approach, with NYVAC followed shortly by Dryvax, may be a safe vaccination strategy.

In immunocompromised macaques that did not receive NYVAC, we found that the $CD4^+$ T-cell count correlated inversely with the size of Dryvax-induced lesions. This observation suggests that the degree of immunosuppression correlates with the severity of Dryvax complications. We also noted that immunization with NYVAC induced neutralizing antibodies to vaccinia virus even in macaques with severe $CD4^+$ T-cell depletion, and that the titers correlated inversely with the time to resolution of a subsequent Dryvax lesion.

SIV infection in macaques reproduces many features of HIV-1 infection. Our findings therefore suggest that a prime-boost approach of NYVAC followed by Dryvax will increase the safety of vaccination against smallpox, and they highlight the potential importance of neutralizing antibodies in protection. Even though such a vaccination strategy may be safe for immunocompromised persons, whether it will also be protective if the person is exposed to smallpox is unclear. We are currently examining whether SIV-infected macaques immunized using the NYVAC-Dryvax prime-boost approach are protected against monkeypox virus, which produces a severe systemic disease similar to smallpox in humans.

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