Outlook

Naval Medical Research and Development Command

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Our mission is to provide timely solutions to Navy and Marine Corps medical and operational problems through biomedical research, development, test, and evaluation

April
1995

INSIDE THIS ISSUE

OUTLOOK ON THE INTERNET

NMRDC is posting the contents of the "Outlook" newsletter - and other items of interest to the NMRDC community - on a gopher-based information system on the Internet. Pending some network and other computer system upgrades, the NMRDC menu is being hosted on the Defense Modeling and Simulation Office's Modeling and Simulation Information System. If you are running gopher, you can connect to the M&S Info System's main menu at 'msis.dmso.mil'. Once connected, select the 'Organizations and Groups' menu item and then the 'NMRDC' menu item. If you have access to Mosaic, or some other Web browser, you can connect to the system's home page at 'http://www.dmso.mil'. From the home page, get to the NMRDC menu by selecting 'MSIS', then 'Organizations and Groups' and then 'NMRDC'. If you have comments on the current NMRDC menu structure, suggestions for items to be added, or know of other information resources which would be of interest to the medical R&D community, let us know. You can e-mail your comments or suggestions to 'rdc03b@nmrdc1.nmrdc.nnmc.navy.mil'.

Dr. Tom Davis from NMRI (left) and Catherine Serke from WRAIR (right) assemble the bone marrow culture system/STL-A in preparation for the Discovery mission.

NMRI SCIENTISTS LAUNCH BONE MARROW CELL GROWTH SYSTEM ON THE SPACE SHUTTLE DISCOVERY

On February 2, 1995 the Space Shuttle Discovery lifted off during a spectacular night launch from the Kennedy Space Center on a nine day mission. The payload included an experiment developed by Naval Medical Research Institute (NMRI) scientists who are investigating the growth and development of bone marrow stem cells.

The research findings of this shuttle experiment can lead, in the future, to healthier and more productive astronauts aboard the Space Station and on a manned-mission to Mars. The data can also lead to life saving techniques on the battlefield for casualties of acute bone marrow injury caused by toxic agents or ionizing radiation.

Kelvin P. Lee, MD, Head of the Stem Cell Biology Branch, NMRI, pointed out, "It has been shown that astronauts exposed to microgravity develop persistent hematologic abnormalities. For example, they become anemic and their lymphocytes don't function normally. The causes are presently unclear, and it is important to know for future missions like the Space Station. If there is ever a Mars Mission with astronauts in microgravity conditions for 18 months or more, researchers will need to predict what's going to happen."

The goal of the space study was to examine how microgravity affects hematopoiesis, the generation of the cellular components of the blood, which occurs within the intricate microenvironment of the bone marrow. Dr. Thomas A. Davis, Associate Chief of the Stem Cell Biology Branch NMRI, and colleagues at NMRI's Immune Cell Biology Program and Cellco, Inc., Germantown, MD, developed a unique in vitro hematopoietic culture system that mimics the bone marrow microenvironment.

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NMRDC COMMAND MASTER CHIEF'S CORNER

by HMCM Cecil McWilliams, USN

Recently, I had the pleasure of traveling to Egypt (NAMRU-3) and have the Force Master Chief (Michael Stewart) address the enlisted population. I can honestly say that I am proud to be serving such a great group of people in Navy Medicine. His praise of NAMRU-3 was fantastic. I boldly told him that all in NMRDC were just as impressive.

In Navy Medicine we talk about the Navy's Core Values, and the enlisted community within the R&D structure practices it:

HONOR: "I will bear true faith and allegiance."

COMMITMENT: "I will obey the orders....."

COURAGE: "I will support and defend..."

In every laboratory I have traveled to, NAVY CORE VALUES ARE PRACTICED.

During the most recent Commanding Officers' Conference, we had most of the senior enlisted attend. We broke away to form our own forum and wrote several point papers concerning enlisted matters. We covered subjects such as communications from NMRDC to the laboratories, awards, TAD's, etc.

I have noticed that some issues have been taken for action by some commanding officers. Any commands needing additional copies of the point papers, let me know. Part of my responsibilities consist of interacting with the detailers on behalf of our enlisted members.

Several points to remember!

Always Keep A Current Enlisted Duty Preference Sheet On File!

Ensure you have a current copy of your microfiche and REVIEW IT for accuracy.

Co-location requests: **PLAN AHEAD**

Military couples desiring co-location must each submit an Enlisted Personnel Action Request (NAVPERS 1306/7) to their detailers 9 - 12 months before PRDs.

Every effort will be made to assign both members to the same geographical location. However, don't ask for orders and assume that your spouse can automatically be assigned to the same area.

Military couples should assess the impact that co-location will have on future duty assignments and plan accordingly.

PLAN EARLY: Geographical location should be of secondary concern since the primary goal is co-location. Co-location requests do not guarantee assignments. Its primary mission is to keep families together.

I Would Like To Take This Opportunity To Say Farewell To

YNCS Steve Rogan, NBDL New Orleans. You did a GREAT JOB and you're leaving big shoes to fill. Good luck in San Diego!

HMCS Ford in NAMRL Pensacola. We wish you a smooth transition into the civilian community. It's always hard to see a shipmate being piped over the side. Thanks for the great job you did.

I can be reached at (301) 295-1095, or at my E-Mail address: 'mcwiliams@mail2.nmri.nnmc.navy.mil'.

Please remember that I'm here to serve you and be your advocate.

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GOT BY THE GATT

by A. David Spevack, Intellectual Property Counsel, NMRDC

Last January, the President signed the General Agreement on Tariffs and Trade (GATT) which placed in motion major changes in the United States Patent System. The changes begin to go into effect midnight, 7 June 1995.

Term

For over 100 years, a U.S. Patent was in force for 17 years from the date it issued. Under GATT, we joined the rest of the world and U.S. Patents will be in force for 20 years from the oldest effective date of the application.

What Does That Mean?

Suppose we file an application on 8 June 1995, and it sits in the Patent and Trademark Office (PTO) for one year before it is examined. Once it is examined, there is an office action requiring a response and we take three months to answer. Then the examiner takes another six months to respond with a second office action. We take three more months to take action and then we refile the application resulting in three more years of prosecution before the patent issues.

The bottom line is that the patent is enforceable for 20 years from the original filing date (8 June 1995) so this patent is only enforceable for 15 years from the date it actually issues.

This is a change from the current case where an application might be filed early and "up-dated" as new information becomes available. There is some provision for restoring part of a lost period under certain circumstances. These relate to interferences and appeals - if Applicants can't be blamed for the delay.

Equal Footing

If the examiner cites a publication, published less than a year before the filing date of an application, as showing that others made the invention before the applicant, the applicant must show that he made the invention first in order to have a valid application.

For many years, foreign inventors have complained that U.S. inventors have the advantage of using their earliest filing date or action in the U.S. to show when they made an invention while foreign inventors can only use their U.S. filing date and not the date of their (earlier) foreign filed applications.

GATT changes that. Under GATT, an inventor can use acts - such as filing in another country - to prove that the inventor made the invention before the publication date of a reference. This is a simplification.

Provisional Applications

The most interesting, and dangerous, aspect of the law is the provisional application. This "application" is simple - pay a small fee and file a description of the invention, which can be anything including the galley proofs of a paper about the invention.

The provisional application is good for one year. Before the year is up, a complete application must be filed. So must any foreign application(s). The application may not protect a later filed invention claiming a broader invention if the provisional application did not provide support for all of the concepts in the later invention. The jury is still out on how useful these provisional applications will be and how they could be used.

A provisional application will save the situation where an article is about to be published and we don't want to lose the right to file a patent application. Publishing information about an invention before an application is filed destroys the right to obtain a patent. More on this subject as the situations evolve.

There is more to come. Under today's law, U.S. applications are kept secret until the patent issues. Congress is considering whether U.S. applications should be published 18 months after filing, as is the case in Europe, even if the application has not been examined - let alone a patent issued. Other laws on infringement and enforcement are coming. Let's see if GATT will be a boon or a GOTCHA.

PHOTOS NEEDED FOR ALL HANDS "ANY DAY IN THE NAVY" ISSUE

Amateur and professional civilian and military photographers are asked to record what's happening on their ship or installation during the 24-hour period of May 18 for a special photo feature to appear in the October edition of All Hands magazine.

Submissions must include full name, rank and duty station of the photographer; the names and hometowns of identifiable people in the photos; details on what's happening in the photos; and where the photos were taken.

Photos must be processed and received by All Hands by June 19, 1995. Mail submissions to: Naval Media Center, Publishing Division, Naval Station Anacostia, Building 168, 2701 S. Capital Street, SW, Washington, DC 20374-5080. Questions may be addressed to PH1 Dolores Anglin at DSN 288-4209 or 202-433-4209 or e-mail anglin@media.navy.mil.

NMRDC SAILORS RECEIVE RECOGNITION

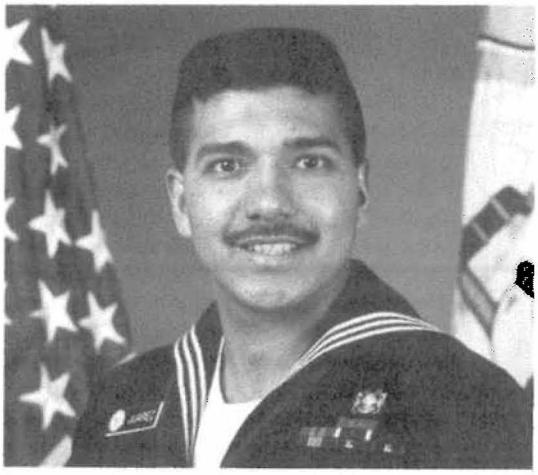
NMRDC SAILOR OF THE YEAR

UT1(SCWS) Geoffrey A. Hittner

UT1(SCWS) Geoffrey A. Hittner has been selected as the Naval Medical Research and Development Command Sailor of the Year. He is assigned to the Naval Medical Research Unit No. 3 (NAMRU-3), Cairo, Egypt, where he is the Public Works Department Assistant Maintenance Control Director. As such, Petty Officer Hittner designed and supervised construction of two emergency egress trainers for Multi-national Forces helicopter pilots. In addition, he ensured completion of repairs and renovation of NAMRU-3 family housing and bachelor quarters, including installation of Armed Forces Radio and Television satellite systems, ceiling fans and air conditioners. As NAMRU-3 Fire Marshall, Petty Officer Hittner conducts fire drill training for 181 employees. In addition, he is the president of NAMRU-3's Petty Officer Association and is actively involved in local community relations.

UT1(SCWS) Hittner is a native of Fremont, Nebraska. He enlisted in the Navy in 1976, and is the father of two children, Kelly Jean and Geoffrey Craig Hittner.

UT1(SCWS) Geoffrey A. Hittner - NMRDC Sailor of the Year



NMRI Sailor of the Year

HM1(DV) Fernando Juarez

HM1(DV) Fernando Juarez was named Sailor of the Year for the Naval Medical Research Institute (NMRI), Bethesda, MD. He is assigned to the Health Monitoring Division of the Diving Research Support Department. Petty Officer Juarez is responsible for maintenance of all official logs and communication systems dealing with the dive chamber. He is a qualified hyperbaric technologist, nationally certified EMT instructor, and emergency vehicle operator and instructor, as well as a volunteer cardiac rescue technician with the Wheaton (Maryland) Rescue Squad.

NHRC Sailor of the Year

HM2 Patrick Wang

HM2 Patrick Wang was named Sailor of the Year for the Naval Health Research Center (NHRC), San Diego, CA. Petty Officer Wang is assigned to the Operating Services Department, where he is responsible for the procurement, receipt and distribution of supplies, as well as maintenance and inventory of plant and property accounts. He is a member of the MWR committee, and is actively involved in the Balboa Tennis Association and the Chinese Tennis Association.

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NMRDC SAILORS RECEIVE RECOGNITION continuted from page 4

NAMRL Sailor of the Year

HM2(AW) Michael A. Cross

HM2(AW) Michael A. Cross was named Sailor of the Year for the Naval Aerospace Medical Research Laboratory (NAMRL), Pensacola, FL. He is a Biological Laboratory Technician, responsible for the installation and maintenance of equipment assets, and assists with the training and research involving eight primates.
Petty
Officer Cross was awarded the Enlisted Aviation Warfare Specialist (EAWS) designator. In addition, he has completed his Associate Degree at Troy State University. He has taught EAWS classes, and is an active member of the Captain's Cup teams at his command.

NDRI Sailor of the Year

HM2 Jeffrey A. Bolden

HM2 Jeffrey A. Bolden was named Sailor of the Year for the Naval Dental Research Institute (NDRI), Great Lakes, IL. He is assigned to the Molecular Biology Scientific Development Department. Petty Officer Bolden is a Leading Petty Officer, assisting in lab research projects, and has implemented an advancement study program for HMs. In addition, he is the Assistant Command Fitness Coordinator and is active in the North Chicago Citizens Against Drugs and Alcohol, and is a member of the County Sheriff's Task Force War on Drugs.

HIGHLIGHTS FROM NAMRU-2

The U.S. Naval Medical Research Unit in Jakarta (NAMRU-2) has announced plans to assist in a study to assess the prevalence of the Hepatitis E virus in the Lao People's Democratic Republic (LPDR), formerly known as Laos.

Working through the U.S. Embassy in Vientiane, LPDR, NAMRU-2 will support a study being carried out by the U.S. National Institute of Hygiene and Epidemiology (NIHE) to determine the importance of the Hepatitis E virus, also known by the initials HEV, as a cause of disease in the LPDR.

In addition to the transfer of study equipment and supplies, the Jakarta-based NAMRU-2 staff will provide training to the LPDR Ministry of Health in the area of laboratory diagnostics and applied epidemiology, as related to the study of HEV. World Vision LPDR will also be participating in activities related to the project.

HEV is now recognized as a major cause of water-borne enteric disease outbreaks, particularly in areas characterized by poor water-related hygiene and sanitation. In 1994, epidemic HEV was identified as the cause of disease for the first time in Indochina in an area south of Vietnam near the border with Kampuchea.

The spread of HEV is a major public health concern given that the virus can cause disease in a large number of people from a single source at the same time. Moreover, it has been found that gamma globulin, an effective therapy in the prevention and treatment of Hepatitis A and B virus infections, is not effective in treating HEV. Therefore, unlike Hepatitis A and B viruses, no vaccine yet exists that is effective against HEV. There is, furthermore, a particularly high death rate among pregnant women infected with the virus. That death rate is estimated to range from 10 to 24 percent of cases.

Project activities will be located in area hospitals around Vientiane and are expected to last approximately twelve to eighteen months.

ANIMAL BYTES

by LTC John Bley, VC, USA, Special Assistant for Veterinary Medicine, NMRDC

"Signs, signs, everywhere signs, messing up the scenery, blowin' my mind. Do this, don't do that! Can't you read the signs?"

When the "Five Man Electrical Band" sang that paean to anarchy 20 plus years ago while I was in Vet School, you would have had an incredulous guffaw thrown back into your face by me if you would have told me that today I would be one of the "suits" - someone responsible for posting and enforcing all those signs. The "Veterinary Police" role that I now fill here at NMRDC is a far cry from the James Herriot (God rest his soul), country Vet image that I had of myself back in those halcyon days.

I won't challenge my credibility further - nor your indulgence - by rationalizing my current position with a cliché such as "somebody's got to do this nasty job" I'll just get right to the bad news and hope that my "spoon full of sugar helps the medicine go down." [Crossed clichés?]

By the time this goes to press, the Deputy Secretary of Defense, John Deutsch, should have signed the new DoD Directive 3216.1 on the "Use of Laboratory Animals in DoD Programs". [Yeah, you've been hearing me promise that the directive's implementation is imminent for months now, but this time ...] It has been a long time coming to the table, but we are overdue for some new direction; the last version of DoDD 3216.1 came out way back in 1982. An updated SECNAVINST 3900.38 will be written just in time for all of our labs to become part of the Armed Forces Medical Research and Development Agency (AFMRDA), but that won't adversely affect us because the Army's instruction (AR 70-18) is the same as the Navy's (which is the same as the Air Force's, which is the same as USUHS's and so on - DoD, remember?).

The impact on our operations will be minimal because most of you have already begun to implement the directed changes. Remember that most of the changes were those that were recognized in last year's DoD IG Reports as "Best Practices", and everyone has been provided with that list already (see your local Vet).

In addition to the DoD Directive, there will soon be a DDR&E and ASD(HA) joint Policy Letter on Animal Use that will underline the directive's changes and require the use of a DoD Standard Protocol Format (beginning 1 October 1995) and a DoD Standardized Semi-Annual Program Review Checklist (again, see your Vet).

For those of you already surfing the Internet, I've posted electronic copies of both the Protocol Format and the Checklist on the NMRDC gopher server. Until we get our own system up and running, the NMRDC gopher menu is located at 'msis.dmso.mil'. When you connect to the main menu, select 'Organizations and Groups' (currently item 4 in the main menu) then select 'NMRDC' (currently item 31 in the organizations and groups menu). If you aren't running gopher or one of the graphical browsers, but do have e-mail, I can e-mail copies of either document. Send me a request at 'rdc00c@nmrdc1.nmrdc.nnmc.navy.mil'.

The directive no longer states that facilities will "seek AAALAC accreditation", but instead says that facilities "shall apply for accreditation". As in RIGHT NOW!!! The good news is that all of the Navy labs have now complied with this, and for that we are both proud of and grateful for the work that you have all done - and continue to do - to meet this requirement.

An alternate to the non-affiliated member of the IACUC is now required of every lab to assure that there is always an outside member at each IACUC meeting. This is a tough one for some of you who have had to scramble to find just one non-affiliated member. And there's more: the non-affiliated members must receive a minimum of eight (8) hours of training in regulatory responsibilities, proper techniques, humane care and animal use ethics. The deadline for this is 1 October 1995.

Commanders of facilities "shall support and, as necessary, develop animal care and use training programs for personnel associated with animal use programs ...". There are many means of accomplishing this requirement, and I welcome your calls for help in developing your own program or borrowing from others.

The somewhat controversial conversion of work unit summaries to a publicly-accessible Internet format will be completed on 1 October 1995 also. Again, the Navy labs have done an outstanding job of completing the call for the FY94 summaries, and after another round of editing, these will be "on the net" as ordered.

A "headquarters-level administrative review" of non-human primate proposals is still required for intramural research. In addition, this Command will continue to review all proposals which use dogs, cats, and marine mammals. If you have any other study that you think might warrant another level of review you may, of course, send those to this office at any time.

Extramural research also receives increased scrutiny under the new directive. All proposals must now be "administratively reviewed" by a DoD veterinarian. Contractors need not use the DoD Protocol Format, but they will be required to include all of the items that are in the format. A contract facility's USDA inspection reports will be reviewed by a DoD Vet annually. And finally, a DoD Vet must conduct site visits of any contractor who is doing DoD-sponsored research using non-human primates, marine mammals, dogs, cats or whose proposals are "deemed to warrant review".

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BONE MARROW CELL GROWTH SYSTEM ON THE SPACE SHUTTLE continued from page 1

Dr Lee said, " Little is known about what actually happens on a cellular basis in the bone marrow at zero gravity. This experiment was one of the first attempts to look at that." NMRI Scientists deployed three sets of human bone marrow cell cultures in low earth orbit. The effects of microgravity on the growth and development of these cells is being examined and compared to identical ground-based controls.

The experiment was an unqualified success. Dr. Lee noted, "Things couldn't have gone much better. We received very positive feedback from the other groups flying experiments on this mission, including scientists from Harvard, NIH, NASA and the Army, regarding the high quality of Navy biomedical research."

According to Dr. Davis, the bone marrow culture system functioned beautifully during the flight. Dr. Davis added, "We are very pleased by how the whole system performed. I think many of our questions regarding the durability of this system for field deployment have been answered."

Initial analysis of the results from this experiment indicate that microgravity decreases the proliferation of bone marrow stem cells compared to ground controls. In addition, there may also be qualitative changes to the stem cells and their progeny grown in microgravity. These findings may point to an important component in the etiology of space flight anemia as well as basic mechanisms in hematopoiesis. Dr. Lee went on to say, "This has been a great experience for us. We hope to pursue our findings on upcoming shuttle missions."

This space shuttle effort was a Navy - Army collaboration. NMRI scientists focused on the medical science of the culture system, where human bone marrow cells are cultured on top of a "feeder" monolayer of microvascular lining cells. The NMRI culture system was used in conjunction with the Army's cell culture unit (the Space Tissue Loss Model A (STL-A)). The STL-A, a self-contained cell culture apparatus, was developed at the Walter Reed Army Institute of Research - Division of Surgery, to be placed in a middeck payload lock on the space shuttle.

Dr. Lee points out, " For military deployments, the bone marrow culture system is a perfect device that can be put in forward areas, because it is small and self-contained. Since it worked in orbit, it will probably work on a hospital ship. The space flight was a great test of whether or not the system will withstand the rigors of field deployment."

NMRI's bone marrow culture system research uses unique Navy resources and seeks to solve problems that are particular to the Navy and the rest of the Armed Forces. Acute bone marrow injury can be caused by battlefield weapons (chemical, biological and radiation weapons), accidental exposure (ionizing radiation from nuclear reactors) or from other injuries (overwhelming sepsis). Development of this bone marrow culture system may result in a forward deployable treatment that is technically simple and no more demanding than a blood transfusion. The system will be portable and will be used for personnel with acute bone marrow injury without the need for a matched donor.

At present, the only effective therapy for severe bone marrow injury is allogeneic bone marrow transplantation from an uninjured donor. This current therapy is severely limited because only 10% of casualties will have a suitably matched bone marrow donor.

Results of this space launch study will aid in the development of new therapies. From a small sample of a patient's own bone marrow, this culture system has the potential of rapidly growing quantities that can be transplanted back into the patient. This kind of treatment, called ex vivo hematopoietic cell expansion with autologous bone marrow transplantation, would be potentially available to 100% of casualties (since their own marrow is used and no donor is necessary) with far fewer complications (i.e. graft vs. host disease) seen with current treatment.

ANIMAL BYTES cont from page 6

Had enough? Me too. Are we any better off with all of these additional "signs" to read? Here, I must stretch your credulity and share with you my very strong belief that most of this should result in a better product once we get over the break-in period of the forms and processes. Do I have any delusions that the grunts in the trenches - the white coats at the benches - share my belief? Not at all.

So my job, just like yours, has become a bit more difficult, but I implore you to lean on me to ease your burden. The current version of the protocol format is now on our gopher (see above) and it can be e-mailed to you or any investigator so that you can do a rough draft, then send it back to me for editing. This should speed things up just a bit, and if you have any other (legal) suggestions, give a call. Until I hear from you, I'll keep "my hair tucked up right under my hat" wondering: why did I get picked to blow this whistle?

FIVE PROPOSALS SELECTED AS NEW STARTS FOR FY96 INDEPENDENT RESEARCH PROGRAM

THE APPLICATION OF IVET (IN VIVO EXPRESSION TECHNOLOGY) TO THE STUDY OF CAMPYLOBACTER PATHOGENESIS

Patricia Guerry, PhD, NMRI, Infectious Diseases Division

In Vivo Expression Technology (IVET) is a genetic method to identify bacterial genes which are expressed only in an animal host. The method was originally developed for Salmonella typhimurium and has led to identification of previously undescribed genes with roles in virulence. This proposal involves (1) development of IVET technology suitable for Campylobacter spp. and (2) application of this technology to the identification of genes required for pathogenesis. The basis of the vector will be a promotorless chloramphenicol acetyltransferase gene (CAT) fused to a reporter gene from C. jejuni whose expression can be monitored on a chromogenic substrate. When genes which are expressed in vivo are cloned upstream of this fusion, they will allow expression of CAT (which renders the cells chloramphenicol resistant) and atsA. When the recombinant pools are used to infect ferrets which are being treated with chloramphenicol, only those clones which express CAT will survive and be able to cause disease. Plating of the surviving recombinants on medium containing the chromogenic substrate will indicate that positive selection has occurred. The color on the chromogenic substrate in vitro differentiates between clones expressed constituitively and those expressed only upon induction in vivo. The characterization of the DNA driving expression in vivo in these recombinant clones should lead to identification of genes and gene products required for Campylobacter pathogenesis. These genes and gene products have direct application to the development of rational vaccine strategies against Campylobacter spp. which represent the most frequently isolated cause of bacterial diarrheal disease worldwide and which have been well documented as a threat to military forces.

STUDIES TOWARDS THE DEVELOPMENT OF AN HLA-DEGENERATE SUB-UNIT VACCINE AGAINST MALARIA

Eduardo Gomez-Saladin, LT, MSC, USNR, NAMRU-2

One approach to malaria vaccine development is to construct a sub-unit vaccine that includes peptide epitopes containing specific binding motifs for a number of MHC molecules. In a broad sense, an allele-specific MHC motif can be defined as the set of structural features of peptide molecules that are recognized by a given type of MHC, allowing high affinity binding to the MHC molecule. A sub-unit vaccine consisting of epitopes recognized by individuals of one HLA type, however, will probably not induce an immune response in individuals of a different HLA type. This problem could be circumvented by incorporating epitopes recognized by a number of HLA alleles which between them would allow for coverage of all racial and ethnic populations. No studies to date have, however, specifically addressed the issue of what percentage of a population of a defined HLA type responds to a given target epitope and how this relates to prevalence of Plasmodium falciparum infection, parasite density and symptoms attributable to malaria. That is the intention of this proposal. Specifically, by defining the prevalence of response of a defined HLA population with exposure to endemic P. falciparum malaria to universal CD4 + T-cell epitopes which bind to multiple class II MHC molecules, we will provide critical information for predicting what proportion of a naive or semi-immune population of a defined HLA type can be expected to respond to vaccination with a peptide known to bind to that specific HLA molecule. Furthermore, by selecting a number of HLA-DR alleles which between them allow for coverage of most of the target population, we will demonstrate the feasibility of a synthetic peptide-based sub-unit vaccine that circumvents the problems associated with genetic variability in human HLA haplotypes. Such an HLA-degenerate vaccine would protect military personnel of any ethnic origin when deployed in malaria-endemic regions.

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INDEPENDENT RESEARCH PROGRAM continued from page 8

SELECTION OF APPROPRIATE TARGETS FOR A POLYVALENT SCRUB TYPHUS VACCINE BY MEANS OF A NOVEL POLYNUCLEOTIDE VACCINE-BASED METHOD

Gregory Dasch, PhD, NMRI, Infectious Diseases Division

No vaccines for scrub typhus are presently available. Age dependent seroprevalence studies have demonstrated that 70% seroconversion rates are frequently observed in endemic areas. In these areas, studies of hospitalized febrile patients indicate scrub typhus may be the major source of illness after malaria. Severe or fatal scrub typhus infections refractory to conventional antibiotic therapy with chloramphenicol and tetracycline antibiotics have been described. Scrub typhus rickettsiae are very diverse antigenically. Homologous protection with live infection has been demonstrated but heterologous protection is short-lived so reinfections occur. The major variable 50-60 kDa protective antigen (VPA) of R. tsutsugamushi has been cloned and sequenced from six antigenic types and four highly variable domains identified. Restriction enzyme typing of over 200 isolates obtained from throughout Asia indicate more than 100 types may be distinguished and that individual types are localized geographically. Selection of isolates for use in a broadly protective vaccine is therefore difficult. Immunization with plasmids ("naked" DNA or polypeptide vaccines) expressing specific antigens has been found efficacious in protecting animal models against viral diseases and malaria. This IR will demonstrate the efficacy of this method for eliciting protection to scrub typhus rickettsiae. Further, it will use this methodology in a novel way to identify essential protective epitopes, to assess the value of cloned conserved non-protective antigens in improving protective activity of the VPA, and most importantly to select VPAs which singly or in combination afford protection to a wide variety of antigenic types of Rickettsia tsutsugamushi. This methodology has the potential to shorten the time necessary for development and fielding of effective polyvalent vaccines against a variety of agents exhibiting extensive antigenic diversity in their proteins.

USE OF IMMUNOLOGICAL TOLERANCE TO SELECT AND MAP VACCINE CANDIDATES

David A. Dean, PhD, NMRI, Immunobiology Division

The induction of specific immunological unresponsiveness (tolerance) represents a razor sharp technique for deleting a single immune response from the millions of possible responses in the total immune repertoire of an animal. In theory, it should be possible to determine whether a particular antigen is required for induction of protective immunity with a complex preparation (such as killed virus or irradiated parasites) by determining whether the induction of tolerance to that antigen reduces the ability of the animal to be protectively immunized. The aims of the IR are to (1) develop procedures for inducing profound, antigen-specific tolerance that persists after multiple immunizing presentations of antigen; (2) design a standard tolerance assay to test for the requirement of specific antigens in complex animal models of protective immunity; (3) use the tolerance assay to test preselected vaccine candidate antigens for importance in animal models of protective immunity; and (4) use the tolerance assay to define minimal immunogenic requirements (epitopes) required for protective immunization. The primary contribution of this project to the Navy and science at large is the development of the tolerance assay, which should accelerate vaccine development. The products expected from this study are 1) the tolerance assay and unambiguous information with respect to candidate requirement: whether a candidate antigen is actually required for the induction of at least part of the protective immunity observed in a given animal model; 2) candidate sufficiency: whether one or more candidate antigens are the only ones required in a model, i.e., are sufficient to account for all of the protective immunity induced; 3) and minimum immunogenic requirements: the minimum molecular structures (epitopes) required for the induction of protective immunity

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VARIATION OF MALARIAL ANTIGENS AND MALARIA ATTACK RATES IN WONJI, ETHIOPIA

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James Campbell, CDR, MSC, USN NAMRU-3

This study will measure the P. falciparum malaria attack rate, density, and parasite variability in adult residents of a village in central Ethiopia during three years. In addition, during the second year of the study the differences in P. falciparum attack rate, parasite densities, parasite variability and clinical manifestations of malaria will be assessed among children less than five years of age with specific attention to HLA type. During each of the three years, 200 adults will be recruited during the expected peak transmission season as a cohort for a six month malaria study. During the second year of the study an additional cohort of children aged 5 and under will be recruited, and HLA typed. During the first two weeks of each cohort study, a radically curative regimen of quinine, doxycycline, and primaquine for adults, or quinine, Fansidar and primaquine for children will be administered to the volunteers. During the ensuing six month, study subjects will be followed prospectively with weekly blood smears and concurrent blood samples on filter paper, and twice weekly questioning concerning symptoms of malaria. Children will also be assessed for hemoglobin levels at time of entry, time of first parasitemia, and at the end of the six month follow up period. Time to first parasitemia will be used to calculate monthly and cumulative malaria incidence rates, and parasite density to establish density at first recurrence. Serological responses to malaria vaccine candidate antigens measured at the beginning of the study will be correlated with time to first parasitemia and density of first parasitemia to look for protective immune responses. Certain candidate antigens from individual waves of parasitemia will be analysed by PCR, and variations over the three years of the study will be documented. Study subjects contracting malaria during the surveillence period will be treated with mefloquine or Fansidar.

NRL RESEARCHERS RECEIVE ALAN BERMAN RESEARCH PUBLICATION AWARD

Two Naval Research Laboratory (NRL) researchers were recently awarded the NRL Alan Berman Research Publication Award for their paper, "Spatially Controlled Adhesion, Spreading, and Differentiation of Endothelial Cells on Self-assembled Molecular Monolayers" which was published in the Proceedings of the National Academy of Sciences, Nov 94.

Barry J. Spargo, PhD, NRL and Alan S. Rudolph, PhD, NRL, along with Thor B. Nielsen, NMRI, and three other researchers co-authored the paper. The underlying research represents a significant step in the ability to control the cellular events on a well defined surface and could have important application in fabrication of smart biomaterials that actively participate in soft tissue regeneration.

The research was supported at NRL by NMRDC Combat Casualty Care and arose out of collaboration between NRL and NMRI investigators.

RETIRED NAMRL CHIEF SCIENTIST HONORED BY AIAA

Dr. Frederick E. Guedry, Jr., who retired as Chief Scientist from the Naval Aerospace Medical Research Laboratory (NAMRL), Pensacola, FL, on 30 November, 1990 received the Jeffries Medical Research Award for 1995 from the American Institute of Aeronautics and Astronautics.

The award, named for the American physician who made the earliest recorded scientific observations from the air, is presented for outstanding contributions to the advancement of aerospace medical research. The citation on Dr. Guedry's award reads, "For outstanding contributions to the advancement of aerospace medical research and operational applications in the field of biophysical and physiological mechanisms of vestibular function, spatial disorientation phenomena, and the psychophysics of vestibular sensation."

Following active service with the Navy in World War II, where he commanded an LST, Dr. Guedry earned his Ph.D. in psychology in 1954. As a research associate during this time, he conducted research at the School of Aviation Medicine - now the Naval Aerospace Medical Research Laboratory - In Pensacola.

He later joined NAMRL in 1961 and retired as Chief Scientist in 1990. In 1968, Dr. Guedry's accomplishments in the psychological aspects of aerospace medicine were recognized by the Aerospace Medical Association with the Raymond F. Longacre Award.

As a consultant to NASA Ames, he helped design and develop the facilities for the Center of Vestibular Research. His contributions to the study of weightlessness and artificial gravity are evidenced by his publications in the NASA-NAMRL series of symposia "The Role of Vestibular Organs in the Exploration of Space." With over 150 research articles on the psychological analysis of acceleration forces published, Dr. Guedry is firmly established as the authority on the perception of acceleration.

TWO NAVY INVENTORS RECEIVE FIRST ROYALTY PAYMENTS FOR LICENSING OF PATENT RIGHTS

(left to right) CAPT Thomas N. Jones, MSC, Commanding Officer, NMRDC; A. David Spevack, patent attorney, NMRDC; Dr. William Kidwell, vice president, research and development, Cellco, Inc.; Dr. Thomas A. Davis and Dr. Kelvin Lee, Immune Cell Biology Program, NMRI and RADM Frederic Sanford, MC, Assistant Chief, Operational Medicine and Fleet Support, BUMED.

Two researchers from the Naval Medical Research and Development Command (NMRDC) received royalty payments for licensing rights of their inventions during a ceremony at the Bureau of Medicine and Surgery. RADM F. Sanford, MC, USN, Assistant Chief, Operational Medicine and Fleet Support formally presented checks to co-inventors Thomas Davis, Ph.D. and Kelvin Lee, M.D. of the Stem Cell Biology Branch, Immune Cell Biology Program at the Naval Medical Research Institute (NMRI), Bethesda, MD.

This license agreement resulted from a Cooperative Research and Development Agreement (CRADA) between the Navy and Cellco Inc., Germantown, MD. This is the first such license granted to a Navy CRADA partner.

A CRADA between NMRDC/ NMRI and Cellco Inc. was established in 1993 for the purpose of developing a deployable stem cell culture system. Three patent applications have been filed covering this technology, one is Navy owned, and two are joint Navy-Cellco owned. Dr. Davis and Dr. Lee are involved in Navy efforts that focus on culturing bone marrow stem cells outside of the body for the treatment of casualties caused by acute bone marrow injury. Bone marrow stem cells give rise to all the mature elements in the blood such as red blood cells, neutrophils, monocytes, lymphocytes and platelets. The natural growth of blood components occurs in the intricate microenvironment of the bone marrow. These inventors have contributed to the development of a unique in vitro culture system that mimics the bone marrow microenvironment.

This unique culture technique is incorporated in a self-contained artificial capillary system, which, when coated with a monolayer of endothelial cells, supports large scale expansion of human stem cells. The system makes the cell expansion procedure very simple, rapid, and cost-effective. The system is currently undergoing qualification for clinical use and is not yet commercially available.

There are important applications of this technology in military and civilian settings for reducing the risks, work-intensity, and cost of bone marrow transplantation and peripheral blood stem cell transplantation. This culture system can be part of life saving techniques for casualties of acute bone marrow injury caused by toxic agents or ionizing radiation.

The Federal Technology Transfer Act (FTTA) allows the government to license government patents for royalties. The FTTA and SECNAVINST 5870.20D require that a percentage of the royalties are shared with the inventors. The remaining royalties are provided to the inventor's laboratory.

HIGHLIGHTS FROM NAMRU-3

Hepatitis E Virus: A Leading Cause of Community-Acquired Hepatitis in Egyptian Adults

A recent study completed by NAMRU-3 investigators demonstrated that Hepatitis E virus (HEV) was the second leading cause of community-acquired acute viral hepatitis in adults in Cairo, Egypt. HEV is endemic in developing countries and is presumably transmitted via the fecal-oral route. Hepatitis E is therefore a disease threat to deployed military personnel.

In the Cairo study of jaundiced patients (18-60 years old) who sought treatment at a public hospital, 31 of 143 (22%) jaundiced patients with elevated liver transaminases (greater than five times normal levels) had acute Hepatitis E.

Acute infections were diagnosed by detecting anti-HEV IgM antibodies by two methods; a western blot assay developed by NMRI and an enzyme-immunoassay modified by NAMRU-3 investigators to detect IgM. The most frequent cause of hepatitis in the study population, hepatitis B virus (HBV), was responsible for 36% of the cases.

HBV infections are preventable by immunization with HBV vaccine. No known risk factors were identified for HEV infection, but HEV-infected patients were younger than the other patients with acute hepatitis. This pattern of sporadic HEV infections in young adults is similar to that reported worldwide for epidemic disease. Studies to develop and evaluate diagnostic tests and to monitor immunological responses following infection are continuing at NAMRU-3.

NAMRU-3 Awards

The U.S. Naval Medical Research Unit No. 3 (NAMRU-3), Cairo, Egypt, was awarded the Meritorious Unit Commendation for outstanding mission effectiveness from January 1992 to April 1994.

CDR James Campbell, Executive Officer of NAMRU-3, was awarded the Meritorious Service Medal (gold star in lieu of second award) for outstanding service as Scientific Director of NAMRU-3.

HMCS Donna Tremblay was awarded the Navy Achievement Medal (gold star in lieu of forth award) for outstanding service as NAMRU-3 Command Senior Chief.

HIGHLIGHTS FROM NDRI

Two Navy Achievement Medals were awarded recently at NDRI

The first was awarded to HM2 James D. Carter for superior performance of his duties while assigned as a laboratory technician in the Immunology Laboratory of the Scientific Investigations Department of NDRI from July 1993 to September 1994. As a result of his dedication and outstanding assistance, a poster presentation was given at the International Association for Dental Research meeting in Seattle, Washington and a scientific manuscript has been submitted for publication.

The second was awarded to DT1 Angel Alvarez for superior performance of his duties while serving at NDRI from January 1994 to January 1995. During that period, he served as the senior enlisted leader and career counselor following the loss of the Command Master Chief and retirement of the only other Chief within the command. These duties were in addition to his normal duties as the Leading Chief Petty Officer for the Clinical Investigations Department.

Two Letters of Commendation were presented recently to NDRI Sailors

HM2 Carolyn Merritt, a laboratory technician in the Immunology area of the Scientific Investigations Department of NDRI, received her letter for her selection as Sailor of the Half Year for the period ending 31 December 1994.

DT3 Lance A. Puckett, an animal caretaker in the Veterinary Services Division, of the Resource Management Department of NDRI, received his letter for superior performance of duties including commendation during a Surgeon General visit. Other duties noted included the initiation of daily study sessions and weekly tests for his shipmates, the organization of the command annual training conference and the organization of a charity drive to provide Christmas gifts to orphaned children in the Lake County area.