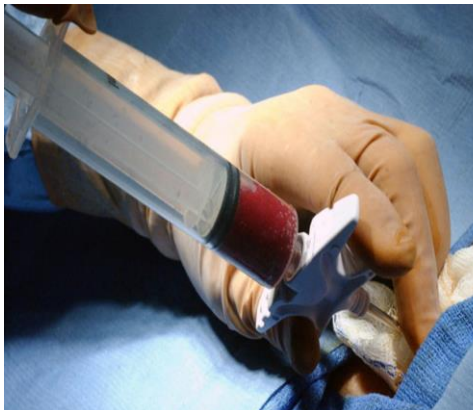
Winter
2014
Volume 7, Issue 1

WEILL CORNELL MEDICAL COLLEGE EDITION

In Focus

Center of Comparative Medicine & Pathology
Research Animal Resource Center
Laboratory of Comparative Pathology



Bone marrow transplantation can result in transplanted immune cells attacking the host's cells resulting in Graft vs Host Disease (GVHD)
Image credit:
http://en.wikipedia.org/wiki/Hematopoietic_stem_cell_transplantation

EnCCoMPass Update

You may have had the opportunity to use various aspects of ***EnCCoMPass***, CCMP's suite of integrated applications developed to streamline many of your interactions and transactions with both RARC and the IACUC. *EnCCoMPass* will initially consist of four modules: ***Protocol***, the animal care and use protocol development and review module; ***Animal*** – an online animal ordering system; ***Census*** – a system to track animals and/or cages and special husbandry requests; and, ***Billing*** – an electronic billing system which provides real time web accessible billing information. *EnCCoMPass* allows you to complete many processes from your workstation, offsite or cageside using a web-connected device. Three modules have been released to date, the release of the last, *Census*, has been delayed due to technical issues resulting in unacceptably long processing speeds users have been experiencing. *EnCCoMPass* version 4.3, released on the 16th of February should improve processing speeds, with further improvement expected when the application is installed on a new dedicated high speed server. CCMP staff overseeing

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Graft-Versus-Host Disease: Inadvertent Induction, Clinical Impact and Endpoints

Graft-versus-host disease (GVHD) is a disease that occurs after transplantation of blood products, bone marrow, and organs[1] Allogeneic hematopoietic stem cell transplantation (HSCT) is a key transplantation therapy used in humans to treat a number of diseases including leukemias and other hematologic malignancies, aplastic anemia, and certain other immune or blood disorders. Successful treatment is dependent upon engrafted donor T cell mediated killing of recipient tumor cells also known as the graft-versus-leukemia effect (GVL). However, donor T cells may also attack other recipient tissues resulting in potentially life threatening GVHD[3].

Mouse and dog models of GVHD have and continue to be instrumental in elucidating the pathophysiology of GVHD as well as in developing new treatments[1,4]. Currently, one of the most common and widely used mouse models of acute GVHD (aGVHD) is a xenogeneic transplant model using NOD SCID gamma (NSG) mice[2]. NSG mice lack

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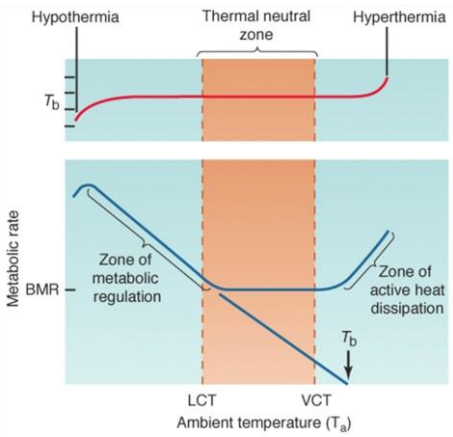
Inside:



THERMONEUTRAL ZONE
The range of environmental temperatures over which the heat produced by a `warm-blooded' animal remains fairly constant, the range in which the animal is `comfortable', having neither to generate extra heat to keep warm nor expend metabolic energy on cooling mechanisms.

Image credit:
http://www.bio.miami.edu/tom/courses/protected/ECK/CH17/figure-17-21.jpg

The weather inside is frightful: How the temperature inside mouse cages may affect research

Several recent studies have investigated how rodent housing temperature can affect different facets of research. Most of these studies focus on the differences between the room temperature recommended by The Guide for the Care and Use of Laboratory Animals (The Guide)[1] and the animals' thermoneutral zone (TNZ). In this article, we will review current housing standards, recent literature suggesting housing temperature as a variable in tumor growth rates, adaptive mechanisms to cold stress, and practical ways of reducing it.

Most research animals are housed in accordance with The Guide[1] that states that "animals should be housed within temperature and humidity ranges appropriate for the species, to which they can adapt with minimal stress and physiologic alteration." The current recommendations for rodent room temperatures are 20-26^{0}C (69-79^{0}F). However, the mouse's TNZ ranges between 26-34^{0}C (79-93^{0}F)[2]. This discrepancy exists to both reduce the risk of heat stress (mice and other rodents are extremely sensitive to hyperthermia) and provide a comfortable working environment for staff. However, these conditions have been reported to have the potential to stunt growth, decrease organ weights, alter immune function, and increase basal metabolic rate[3].

Several recent papers have shown that these housing conditions can also influence tumor growth rates. Kokolus *et al* demonstrated in a recent PNAS paper that housing mice at temperatures below their TNZ increases tumor growth rates by altering the adaptive immune response which in turn reduces tumor suppression[4]. The authors' principal findings were that various tumors (implanted and carcinogen induced) grew at an accelerated rate and had higher incidences of metastases in BALB/c and C57BL/6 mice housed at temperatures recommended in The Guide, compared to those housed at

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Meet Jose Cardona Costa, Ph.D. Manager, Aquatics Systems and Services

EnCCoMPass Census New Features

- View your (and your staff's) cage inventory in real time.
- Reassign cages to different lab members permanently or temporarily if on vacation or away from the College.
- Search for active and historical* cages by protocol, funding source, assigned lab member, location, etc.
- Assign internal or external funding sources by cage to pay per diem.
- Change funding source for specific cages online at any time.
- Generate your own barcode labels** for cage cards.
- Place and track Special Husbandry Requests online.
- Receive email notifications for dead animals, the initiation of Special Husbandry Requests, and the identification of overcrowded and grossly overcrowded cages.
- Electronically store unique laboratory associated data with each cage that can be retrieved cageside or from any web-connected workstation or device.
- Split cages generating new cage cards and transferring cage information to daughter cages providing database access to historical cage relationships.
- Designate a staff member(s) to serve as Lab Manager to be copied on all *EnCCoMPass* email notifications for selected individuals on a specific protocol.
- Generate reports providing current and historical census data with various filters.

*On or after Nov 1, 2013. eCensus data was not migratable.
** Using a desktop printer and Avery TEMPLATE 6871 label sheets.

Jose Cardona Costa Joins the CCMP

The Center of Comparative Medicine and Pathology (CCMP) is pleased to welcome Dr. Jose Cardona Costa, as the new Manager, Aquatics Systems and Services. Jose received his Bachelor of Science in Agricultural Engineering from the Polytechnic University of Valencia (Spain), specializing in Zootechnics. He received his Masters in Animal Science and his PhD degree in Animal Husbandry from the same university in 2010. Soon after graduating, Jose started a postdoctoral training program at Yale University where he also managed the Zebrafish Core Facility for the Yale Cardiovascular Research Center for two years. After he completed his training he remained at the center as an Associate Research Scientist. Jose is passionate about all aspects of husbandry and management of aquatic species. He has more than 10 years of experience working with the zebrafish model and its husbandry, including the management of aquaculture facilities in academic research settings. Jose has authored several research publications in his areas of expertise including cryobiology and micromanipulation techniques in zebrafish.

for Survival Repetitive Hematology and Blood Chemistry Analysis in Mice

The LCP can now provide comprehensive hematology and clinical chemistry profiling in all laboratory species with blood volume requirements low enough to facilitate survival sampling in the mouse. CBC testing, including an automated WBC differential and reticulocyte count, requires only 40 uL of EDTA whole blood. Our Rodent Chemistry Panel consisting of 20 directly measured chemistries (enzymes, electrolytes, and other critical metabolites) requires only 120uL of serum. The total blood volume required for both test disciplines is under 300uL. This capability provides a more comprehensive health status of individual study animals, simplifies experimental designs and can significantly reduce animal usage.

No need for OJ post phlebotomy. Minimal blood requirements aid survival and provide the LCP with enough sample for maximum results

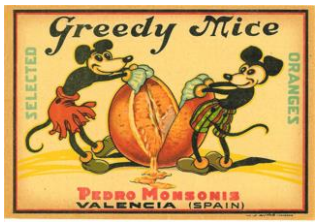

Image credit: http://farm3.static.flickr.com/2218/2472861583_63e4e3d0e4.jpg?v=0

EnCCoMPass Update,

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the *EnCCoMPass* rollout continue to monitor the situation and Census will not be released until the problem is resolved. See left margin for a select list of ***EnCCoMPass Census*** capabilities.

Importantly, the implementation of the *Census* module will be associated with changes in a number of CCMP's business practices and operational processes with which users must be familiar. Highlights of these changes are as follows:

- ***EnCCoMPass*** requires that a **specific animal user be assigned to each cage** maintained in the facility. The animal user will receive all email notifications[1] pertaining to that cage, including dead animal, and overcrowded and grossly overcrowded cage condition notifications. This individual will be contacted in case of animal health, regulatory or husbandry issues associated with a specific cage.
- **Census** requires a unique funding source to be assigned by cage against which per diem will be charged.
- **Census** tracks individual cages (using the cage's unique bar code) and uses the information to calculate per diem charges. This is distinct from the prior method which utilized the total number of cages present, but did not consider each cage as a unique entity for billing. Cage cards **MUST NOT** be reused as the laboratory may be billed retroactively for the period in which the cage card was not associated with a cage within the facility. Cages must be **activated or deactivated** when a new cage is created, or conversely, when a cage is no longer in use. Cage activation and deactivation can be performed at the desktop or cageside using a hand-held, web-connected device.
- The process for requesting and implementing **Special Husbandry Requests (SHRs)** will change. SHRs will be submitted electronically through EnCCoMPass and SHR cards will be unique to each cage. SHR cards **must be activated and deactivated** as described above for cages.

Online interactive training modules and written instructional guidelines are available for each of the modules that are accessible on our web site. We appreciate any and all feedback relating to both your positive and negative experiences with *EnCCoMPass* as well as suggestions for future enhancements. Please email us at enccompass-support@med.cornell.edu

1. E-mail notification preferences can be set within *EnCCoMPass*. Opt-out functionality is provided for many notification types.

Graft-Versus-Host Disease, *Cont. from pg. 1*

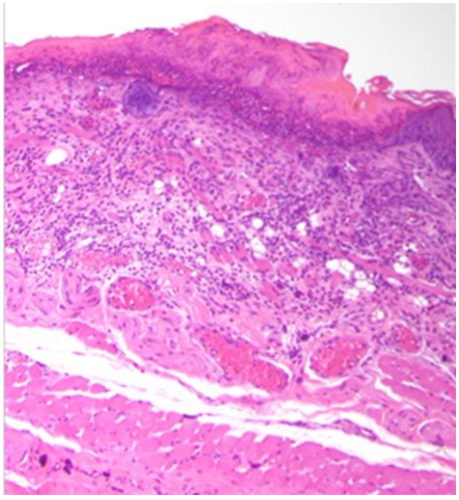

Figure 1: Photomicrograph of a hematoxylin and eosin stained section of the skin of an NSG mouse with xGVHD. Histopathological findings include lymphocytic infiltration, small epidermal erosions and ulcerations, epidermal hyperplasia, and hyperkeratosis.

Figure 2: NSG mouse displaying clinical signs of xGVHD including scaly, itchy, and erythematous skin as well as ruffled fur and alopecia.

"Implementation of humane endpoints and recognizing clinical signs requires a thorough knowledge and understanding of the characteristics of a healthy rodent with normal behavior."
Refer to the following website for further information:
http://www.humane-endpoints.info/eng/index.php?option=com_content&view=article&id=51&Itemid=58&lang=en

mature B and T cells as well as functional NK cells. They also have reduced dendritic cell and macrophage function as well as deficient cytokine signaling[2,5]. In this model, in addition to their baseline genetic immunosuppresion, NSG recipient mice are often first conditioned via sublethal irradiation which ablates their bone marrow to allow for better engraftment[2,6.] However, irradiation also leads to a cytokine storm which influences subsequent development of GVHD via activation of donor T cells and other immune cell types[2].

It should be noted that this mouse model of aGVHD has different underlying pathophysiologic mechanisms than human aGVHD. In humans or allogeneic models, donor T cells are activated by recipient antigen presenting cells (APCs) via major histocompatibility complex (MHC) presentation of recipient peptides (MHC I activating CD8+) and exogenous peptides (MHC II activating CD4+)[2].

In the xenogeneic mouse model of aGVHD (or xGVHD), human T cell receptors (TCRs) do not recognize mouse MHC, therefore human APCs are needed to present mouse antigens to human CD4+ T cells which leads to a T-helper (Th) 1 response[2]. After xenogeneic transplantation of peripheral blood mononuclear cells (PBMCs), mice develop an aGVHD-like disease causing death in approximately 3-7 weeks[2.] Histopathology has shown that T cell trafficking and infiltration is consistent between mouse models and human aGVHD with T cell cytotoxicity noted in the skin, intestines, liver, kidneys, lungs, thymus, and secondary lymphoid organs (Figure 1[1,2]).

While some investigators endeavor to study GVHD through models like the xenogeneic GVHD (xGVHD) NSG mouse model, other investigators inadvertently induce GVHD. Currently, some investigators are testing PBMC treatment modalities in NSG mice after successful xenografting (following irradiation) of human tumor cell lines. Even though these studies are focused on human T cell (or other human immune cells) immunotherapy of cancer, they are usually complicated by the development of xGVHD. Clinical signs associated with xGvHD include scaly, itchy and erythematous skin, especially around the eyes and ears, as well as ruffled fur, alopecia, weight loss, hunched posture, decreased activity level, and a generally deteriorating body condition (Figure 2). Although the diagnosis of this condition is self-evident given the experimental history, corynebacterium associated hyperkeratosis (CAH) must be ruled out due to similar clinical presentations involving the skin. CAH should resolve with antibiotic treatment, such as amoxicillin medicated feed, after a few weeks. If clinical signs do not resolve, a presumptive diagnosis of xGVHD can be made. A definitive diagnosis of xGVHD can only be made via a post-mortem histopathological evaluation.

Unfortunately, there are currently no effective treatment options available for GVHD[2]. However, a recent study has shown that enriched regulatory T cells significantly delayed death due to xGVHD in mice[3]. Therefore, if GVHD is an expected outcome, whether intentional or inadvertent, mice should be examined regularly using a clinical scoring system so that humane endpoints can be appropriately and consistently established. For example, one study examining T cell responses in mice with GVHD assessed the animals every other day scoring them on a scale from 0-3 for each of the following: weight loss, posture, activity, fur texture, and skin integrity[7]. Mice with combined scores greater than or equal to two were scored daily and mice with combined scores greater than or equal to six were considered to have reached the endpoint and were euthanized[7]. A similar scoring system should be employed which balances the research needs with the need to establish humane endpoints.

Clearly there is much work to be done continuing to elucidate the pathophysiology of GVHD as well as developing new transplantation treatment modalities. Whether investigators are directly studying GVHD or inadvertently inducing it, they should be aware of the clinical sequelae and institute appropriate endpoints.

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The weather inside is frightful, *Cont. from pg. 1*

RARC employs a variety of environmental enrichment products providing shelter as well as the opportunity for rodents to exercise innate behaviors. From upper left- Neslets™, made from virgin cotton and Envirodri® dust free paper.

Image credits: http://www.ssponline.com/index.htm and http://www.northkentplastics.co.uk/accessories.asp

Group housing, along with providing the social atmosphere mice crave, is a necessary thermogenic mechanism.

Image credit: http://en.wikipedia.org/wiki/File:Fancy_mice.jpg

A novel way to keep mice cozy?

Image credit: http://ugly-sweater.blogspot.com/2011_12_01_archive.html

temperatures within their TNZ. The increased tumor growth rates correlated with a decrease in CD8+ T cells and an associated decrease in IFN-γ production, both of which are important regulators of tumor suppression.

In contrast, another study found blunted tumor growth in conditions of chronic cold stress[5]. David et al compared Prdkcscid and Nu-Foxn1Nu mice with implanted human epidermoid carcinoma cells housed in static and individually ventilated cages (IVC) with and without plastic shelters. Their results showed reduced rates of tumor growth in mice with indicators of chronic cold stress. They also found that mice housed in IVC without shelter exhibited higher cold stress, and consequently slower tumor growth, compared to mice housed in static cages.

Mice adapt to cold in several ways. The major mechanisms include behavioral changes, insulative responses, and thermogenesis[5]. Behavioral mechanisms include use of shelters, burrowing, nest building, and huddling with conspecifics. The insulative response is change in and prioritization of blood flow towards vital organs and away from peripheral tissues. Thermogenesis is primarily accomplished by increasing metabolic activity of brown adipose tissue[6]. These factors are especially relevant for mice used in tumor studies, as tumors may amplify cold stress[3].

Current housing practices already take into consideration the potential for cold stress in rodents, and The Guide recommends nesting material and group housing be provided. This not only promotes social behaviors, but also supports behavioral thermoregulation. Nesting material, in particular, has been shown to provide partial relief from chronic cold stress[7]. The aforementioned studies also found that mice have fewer temperature dependent effects on tumor growth rates when provided with shelter[5]. That being said, various nesting materials are used across institutions, including but not limited to plastic shelters, paper and cotton products. When housed at 20-22^{0}C, mice seem to prefer fibrous materials they can manipulate over rigid housing structures[8]. This is consistent with the nesting materials provided at MSKCC and WCMC.

In conclusion, several recent studies suggest that the current housing temperatures may be influencing the growth rate and metastasis of various tumors. Unfortunately, their inter-study variability, including different tumor cell lines, mouse strains, types of nesting materials, and caging type, makes it difficult to draw consistent conclusions. These are just the first of what are sure to be many studies examining the effects of temperature and housing on rodent cancer models. Until there are more consistent and conclusive results, mice will continue to be housed in accordance with The Guide, as is current practice at MSKCC and WCMC. In addition, these studies highlight the importance of accurately describing caging type, nesting materials used, and group housing status in future rodent cancer model publications.

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