実験動物ニュース

The Japanese Association for Laboratory Animal Science

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Vol. 57 No. 1 / January 2008

他　学　会　情　報

ICLAS情報

1. 関連学会, 講習会等の案内

a. NABR's 2008 Leadership Conference and International Forum, Communication & Collaboration: An International Forum for Animal Research Policy

June 23–25, 2008, Washington, DC

For more information, see http://www.animalresearchforum.org/.

b. Alternatives and 3Rs Workshop

January 24–25, 2008 in Los Angeles, sponsored by UCLA and the Johns Hopkins Center for Alternatives to Animal Testing (CAAT). Objective of the workshop is to learn about new scientific innovations and ethical perspectives in research animal alternatives and the "3Rs". Please contact Kathy Wadsworth at arc@oprs.ucla.edu if you request additional information.

c. ACLAM Foundation

The ACLAM Foundation Committee members are pleased to announce our solicitation of research proposals in Laboratory Animal Science and Medicine.

For more information, go the aclam foundation website:

http://aclam.org/foundation/rfp.html

or contact:

Dr. Gregory P Boivin

Scientific Director, ACLAM Foundation

University of Cincinnati

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d. 29th World Veterinary Congress

July 27–31, 2008

Vancouver, Canada

Supported & Promoted by CVMA, World Organization for Animal Health and World Veterinary Association

<http://www.meet-ics.com/wvac2008/support/footer.jpg>

CONGRESS SECRETARIAT: International Conference Services Ltd., Suite 2101-1177 West Hastings Street, Vancouver, BC Canada V6E 2K3

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2. ニュース

Dr. Joe Held逝去のお知らせ

ICLAS is saddened to announce the death of Dr. Joe Held. Dr. Held was an inspiring leader in laboratory animal science in North and South America and throughout the world. His contributions to the advancement of laboratory animal science in Latin America were significant. He was instrumental in developing laboratory animal resources at the U.S. National Institutes of Health and extended those resources to the activities of the PanAmerican Health Organization. Among his many contributions, he was chairman of the International Scientific Advisory Board of the Institute for Primate Research, National Museums of Kenya. He chaired the committee of the Council for International Organizations of Medical Sciences (CIOMS) responsible for drafting CIOMS's "International Guiding Principles for Biomedical

Research Involving Animals". He and Dr. Tatsuji Nomura instituted the US-Japan meetings, held annually to discuss salient issues in the use and quality of animals required for research.

Dr. Held was a friend of ICLAS and we honor his many contributions throughout the world. We send our sincerest condolences to his wife and family.

3. 出版

(2004) Guidelines for the Use of Fishes in Research. These are available at the American Fisheries Society.

web page: http://www.fisheries.org/afs/publicpolicy/guidelines2004.pdf

4. ICLAS会議

a. ICLAS International Consortium Meeting

2007年10月16日, 米国North CarolinaのCharlotteでAALASと合同の上記会議が開催されました。その議事録によりますと, 参加者は12カ国, 25名, 日本からは黒澤先生が参加されました。その主要議題を以下に示します。

1. New ICLAS Governing Board (2007–2011)
2. Harmonization of Guidelines update.
3. Reciprocal Registration
4. ICLAS Network for the Promotion of Animal Quality in Research update
5. Americas Committee update
6. ILAR Update of the Guide for the Care and Use of Laboratory Animals
7. Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) update.
8. Announcements and Future Meetings

b. ICLAS FYI Bulletin

ICLAS FYI Bulletinの受信者を更新中です。受信希望者は氏名, メールアドレスを送ってください。

I am in the process of updating the list of recipients of the ICLAS FYI Bulletin. Please let me know if you wish your name to be removed or if you would like to have individuals added. Please send me names and email addresses if you wish to add colleagues to the list.

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The Guidelines of International Nomenclature

The information of animals, strains, genes, etc, appearing in papers submitted to the official journal Experimental Animals, should be given according to the rules and guidelines of international nomenclature. For this reason, authors are required to implement the nomenclature. The editorial board advises authors to refer to the information below, when preparing a manuscript for submission to Experimental Animals for publication.

The current nomenclature applied to mice and rats follows the rules and guidelines established by the *International Committee on Standardized Genetic Nomenclature for Mice*. This nomenclature will be applied to other experimental animals, too.

1. Scientific name of experimental animals

The scientific name of animals used in animal experimentations should be given.

Examples:

a. Mouse: *Mus musculus musculus*

b. Rat: *Rattus norvegicus*

2. Breeder's name or Institute's name

The official title of the Breeder or Institute from which the experimental animals originated or were purchased should be given.

a. Use of "in-house" or "domestic", is recommended, if the animals originate from the author's animal facility.

b. The official title should be referenced from the home page or brochure (pamphlet) issued by the Breeder or Institute.

3. Strain name

(1) Names of strains distributed by commercial breeders should be referenced from the Breeder's home page or brochure (pamphlet).

(2) Names of newly bred strains should be given according to international nomenclature and international index of experimental animals.

Home page of Rules and Guidelines for Nomenclature of Mouse and Rat Strains:
http://www.informatics.jax.org/mgihome/nomen/strains.shtml
http://rgd.mcw.edu/nomen/nomen.shtml#StrainNomenclature

Home page of the Index of Major Mouse Strains:
http://www.informatics.jax.org/external/festing/mouse/STRAINS.shtml

Home page of the Index of Major Rat Strains:
http://www.informatics.jax.org/external/festing/rat/STRAINS.shtml
http://rgd.mcw.edu/strains/

4. Genes, Genetic Markers, Alleles, and Mutations

Scientific and formal names of genes and proteins should be found in Mouse Genome Informatics (MGI) and Rat Genome Database (RGD).

MGI home page: http://www.informatics.jax.org/mgihome/nomen/gene.shtml

RGD home page: http://rgd.mcw.edu/nomen/nomen.shtml#StrainNomenclature

5. Laboratory code (Lab code)

A Laboratory Code is a code of usually one to four letters (first letter is upper case, followed by lower case letters), that identifies a particular institute, laboratory, or investigator that produced, and may hold stocks of a DNA marker, an animal strain, or a mutation. Laboratory codes are also used in naming chromosomal aberrations and transgenes.

Examples:

(1) C3H/HeH: Mouse substrain derived at Harwell (H) from the Heston (He) substrain of C3H

(2) D8Mit17: A DNA segment that has the 17th locus mapped to mouse Chromosome 8 by MIT *Institute for Laboratory Animal Research (ILAR)*

Home page for search: http://dels.nas.edu/ilar_n/ilarhome/search_lc.php

Home page for registry: http://dels.nas.edu/ilar_n/ilarhome/register_lc.php

6. Miscellaneous

(1) Full translations and comprehensive explanations of nomenclatures translated to Japanese are available at the following address.
http://cardb.cc.kumamoto-u.ac.jp/transgenic/index.jsp

(2) “Godfather” as a naming tool is available at the following address for learning nomenclatures.
http://www.shigen.nig.ac.jp/tools/godfather/index.jsp?lang=japanese

Note

The implementation of the nomenclature is carried out at the following databases, MGD (Mouse Genome Database), IMSR (International Mouse Strain Resources), CARD (Center for Animal Resource and Development at Kumamoto University), BRC (RIKEN BioResource Center), EMMA (European Mouse Mutant Archive), RGD (Rat Genome Database) and NBRP-Rat (National Bio Resource Project for the Rat) through each Genetic Nomenclature Committee.

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原著

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低侵襲性の免疫法として有望である経鼻免疫による狂犬病発症防御免疫誘導の可能性について検討した。経鼻免疫抗原には，不活化狂犬病ウイルスを超遠心濃縮したもの（CRV）を用いた。CRV 30 μlに粘膜免疫アジュバントであるコレラ毒素（CT）5 μgを添加し，6週齢雌ddYマウスに5回経鼻投与した。また，対照群として，CT非添加経鼻免疫群，および同量のCRVを2回腹腔免疫した群を設定した。CT添加経鼻免疫群の抗原特異的IgG抗体価は腹腔免疫群と同程度であった。また，CT添加経鼻免疫群の狂犬病ウイルスRC-HL株に対する中和抗体価は，腹腔免疫群より若干低値を示した。一方，CT非添加経鼻免疫群の特異的IgG抗体価および中和抗体価は，前述の2群よりも有意に低値であった。さらに，CT添加経鼻免疫群と腹腔免疫群では，特異的IgG_{2a}抗体価が高値を示し，Th1細胞の活性化が示唆された。免疫マウスに致死量の狂犬病ウイルスCVS株を接種したところ，CT添加およびCT非添加経鼻免疫群のそれぞれ67%および17%が生残し，腹腔免疫は全頭生残した。生残マウスと死亡マウスの間には特異的IgGおよびIgG_{2a}抗体価，中和抗体価に有意な差が認められた。これらの結果は，不活化狂犬病ウイルスを用いた経鼻免疫による狂犬病発症防御免疫の誘導が可能であること，さらにCTの添加によりその効果が増強されることを示唆している。

Sunhee SHIN[1)], Ja Young JANG[1)], Byong-il CHOI[1)], In-Jeoung BAEK[1)], Jung-Min YON[1)], Bang Yeon HWANG[2)], Dongsun PARK[1)], Jeong Hee JEON[1)], Sang-Yoon NAM[1)], Young Won YUN[1)], and Yun-Bae KIM[1)]

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The effect of water extract of licorice (*Glycyrrhiza uralensis*), one of the most widely used medicinal plants in Oriental nations and in Europe, on male reproductive function was investigated in rats. Licorice extract was prepared as in Oriental clinics and orally administered at doses of 500, 1,000 or 2,000 mg/kg, the upper-limit dose (2,000 mg/kg) recommended in the Toxicity Test guideline of the

Korea Food and Drug Administration, to 6-week-old male rats for 9 weeks. Licorice extract neither induced clinical signs, nor affected the daily feed consumption and body weight gain. There were no significant changes in testicular weights, gross and microscopic findings, and daily sperm production between vehicle- and licorice-treated animals, in spite of slight decreases in prostate weight and daily sperm production at the high dose (2,000 mg/kg). In addition, licorice did not affect the motility and morphology of sperm, although the serum testosterone level tended to decrease without significant difference, showing a 28.6% reduction in the high-dose (2,000 mg/kg) group. The results suggest that the no observed adverse-effect level of licorice extract is higher than 2,000 mg/kg, the upper-limit dose, and that long-term exposure to licorice might not cause profound adverse effects.

Yea Eun LEE[1)], Sang Kuk BYUN[1)], Sunhee SHIN[1)], Ja Young JANG[1)], Byong-il CHOI[1)], Dongsun PARK[1)], Jeong Hee JEON[1)], Sang-Seop NAHM[2)], Jong-Koo KANG[1)], Seock-Yeon HWANG[3)], Jong-Choon KIM[4)], and Yun-Bae KIM[1)]

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The present study was conducted to elucidate the susceptibility of embryos and fetuses at different gestational stages to the maternal stress in mice. Groups of pregnant ICR mice were subjected to daily 12-h restraint stress, taped in the supine position on a plastic board, on gestational days (GD) 1–4, 5–8, 9–12 and 13–16, respectively. Caesarean sections were performed on gestational day 18, and the fetuses were weighed and examined for morphological defects. During the daily restraint for 4 days, the maternal body weights markedly decreased. Although the body weights recovered gradually after termination of the stress, the recovery was not full until the final stage of pregnancy. Interestingly, restraint stress caused growth retardation of the fetuses, leading to a significant decrease in their body weights, and increased early and late resorptions of embryos and fetuses according to the stress periods. Although the preceding (GD1–4) and concurrent (GD5–8) stresses did not affect embryonic implantation, restraint stress on GD9–12 caused cleft palate. Whereas vertebral abnormalities, mainly bipartite ossification, were observed only in animals stressed on GD5–8, abnormalities of sternebrae, exhibiting asymmetric or bipartite ossification, were enhanced by the stress at all of the gestational stages. On the other hand, the incidence of other malformations including renal malposition and costal abnormalities was not increased by stress at any of the 4 stages. Taken together, the results suggest that intensive restraint stress influences the maternal body weight resulting in growth retardation and increased mortality of embryos and fetuses, in addition to gestational stage-specific ventricular dilatation, cleft palate and sternal abnormalities.

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The aim of this study was to evaluate the effects of progesterone and ovarian stimulation on the development and implantation rate of mouse embryos. Two-cell embryos were collected from superovulated mice and cultured in the presence of different concentrations of progesterone (0, 5, 10 and 20 ng/ml). Also other mice were rendered pregnant in unstimulated, unstimulated progesterone-injected, superovulated and superovulated progesterone-injected groups to collect the blastocysts. The number of blastocysts and implantation sites were recorded on the 4th and 7th day of pregnancy, respectively. The diameter and cell number of blastocysts were analyzed in the *in vitro* and *in vivo* groups. After 120 h culture, the percentage of hatched blastocyst embryos in control and 5, 10 and 20 ng/ml progesterone-injected groups were 63.9%, 64.2%, 64.2% and 75.6% respectively. There were significant differences between the developmental rates of embryos in the presence of 20 ng/ml progesterone and the control and other concentrations of progesterone-injected groups ($P \leq 0.001$). The in vivo blastocyst survival rate (97.68%) and implantation rate (92.06%) in the unstimulated and progesterone-injected groups were higher than in the other groups. Blastocyst cell numbers in the superovulated (128.62 ± 1.30) and superovulated progesterone-injected groups (126.88 ± 1.60) were significantly different from the control ($P<0.001$). The progesterone injection without ovarian induction improved the embryo survival and implantation rates, but after superovulation it did not ameliorate the negative effects of superovulation on the implantation rate.

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メタンフェタミン（methamphetamine：MAP）の投与回数が免疫反応におよぼす影響について雌雄マウス（ddY）を用いて検討を行った。マウスの腹腔内にMAP 5 mg/kg/日を1，5および10回の投与を実施し，白血球数，脾リンパ球のマイトゲン反応（phytohemagglutinin：PHA，lipopolysaccharide：LPS）およびnatural killer（NK）細胞活性について検討を加えた。また，雌雄マウスの脾臓内MAP濃度とその代謝過程で産出されるアンフェタミンの濃度を経時的に測定した。その結果，MAPの単一ならび複数回投与で雌雄ともに白血球数の減少が認められた。PHAに対する反応では，単一投与で雄のみに明らかな上昇が認められ，LPS反応では雌雄とも僅かな上昇がみられた。NK活性では雌雄とも活性の低下がみられ，特に雌では5回の投与後に活性が消失した。しかし，10回の投与後にNK活性と白血球数は対照群のレベルまで回復する傾向がみられた。脾臓内のMAP代謝は雌で遅延する傾向が認められた。これらの結果より，MAPの免疫機能に対する影響は，投与量のみならず投与回数や雌雄によっても変化する可能性が示唆された。このような免疫反応に対するMAPの基礎的な検討は，中毒者の免疫系の変化を理解する上で必要であると思われる。

Dendritic cells (DCs) are the most potent antigen-presenting cells (APC) of the immune system, and are critically involved in initiation of immune responses in autoimmune diseases. They can modulate the nature of immune responses to stimulatory or tolerogenic fashion. Previous studies have demonstrated that the administration route of DCs is an important variable in eliciting anti-tumor immunity. In this study we used experimental autoimmune encephalomyelitis (EAE) as an animal model of multiple sclerosis to compare different protocols of DC delivery in autoimmunity or tolerance induction. Dendritic cells were generated from bone marrow cells of C57BL/6 mice by culturing in the presence of GM-CSF and IL-4 for 7 days, followed by 2 days culture with TNF-alpha. The obtained DCs were pulsed in vitro with myelin oligodendrocyte glycoprotein (MOG) peptide and injected ($5x10^5$ cells/mouse) via the intravenous (i.v.), intraperitoneal (i.p.) or subcutaneous (s.c.) route into female C57BL/6 mice. In some instances pertussis toxin was also injected zero and 48 hours after DC injection. After follow up of the mice pretreated in this way for 4 weeks, in the i.v. group in which no clinical signs of EAE occurred, the mice were immunized with MOG peptide for EAE induction via the common method and the results were compared with mice that were not pre-immunized. Only after three s.c. DC injections with pertussis toxin, the mice showed mild clinical signs of EAE, whereas mice given i.v. or i.p. injections with or without pertussis toxin failed to develop EAE after 4 weeks. Induction of EAE via the common method after three injections of TNF-alpha treated DCs, in i.v. injected groups showed no protection from EAE. It seems that several factors influence the tolerance versus immunity induction by DCs. Our results showed that the administration route of DCs is one of the pivotal factors in DC-based induction of autoimmune diseases.

P. pneumotropica（Pp）とラットから分離されたV因子要求性 *Pasteurella* 科菌（VFDP）の免疫不全ラットに対する病原性を調査するためにそれぞれの菌株3株（ATCC 35149, CNP 160, RPZ）と4株（V6–V9）を用いF344-*rnu* ラットに対し感染実験を行った。4匹1群の動物に各株菌液を二回（0日目, 14日目）経鼻接種し, 初回接種後60日, 120日に剖検, 細菌学的および組織病理学的検索を行った。Pp株を接種した動物では31日目までにATCC 35149株とCNP 160株を接種したそれぞれ数匹の動物にくしゃみの症状が観察された。細菌学的検索では60日目に主に鼻腔, 気管, 120日目に鼻腔, 気管, 肺から菌が分離された。組織病理学的検索ではATCC 35149株とCNP 160株を接種した全ての動物の鼻粘膜上皮に炎症・壊死像が観察された。RPZ株を接種した動物からは臨床上の異常症状も組織病理学的な異常所見も観察されなかった。VFDP株を接種した動物の細菌学的検索では60日目に主に気管, 120日目に気管と肺から菌が分離されたが, 全ての動物で臨床上異常な症状と組織病理学的に異常な所見は観察されなかった。その結果, Ppは株により免疫不全ラットへの病原性が異なる事が示された。またVFDPはラットの呼吸器に寄生する非病原性の細菌だと思われた。

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テイルフリック反射は脊髄反射と考えられているが, 上位中枢からの影響も受けている。テイルフリックテストは痛覚の測定にもっとも用いられている方法であるが, 熱源の特性と繰り返し試験における測定値の信頼性について記述された報告は少ない。本研究では, 上位中枢からの影響を抑制する浅麻酔下におけるテイルフリックテストでの有用性とテイルフリック潜時に影響を及ぼす放射熱の皮膚面温度への経時的効果について検討した。はじめに0, 0.5, 1.0, 1.5%イソフルラン吸入麻酔下での1時間の繰り返しテストにおける潜時への影響について検討し, つぎに熱刺激装置の赤外線（IR）放射熱量の特性を測定し, 非線形近似式によって尾皮膚温の変化を推定し, 再現性を得るための設定について検討した。この結果, 吸入麻酔は濃度依存性にテイルフリック反射を抑制した。またIR放射熱量は2.5秒以後に一定になり, 皮膚温は最大IR放射熱量と放射時間に依存した。1.0%イソフルラン吸入ならびに10秒後に65℃となる161.5 mW/cm^{2}の放射熱量による刺激強度によって再現性のある潜時が得られた。本研究の結果は, テイルフリックテストにおいて再現性のある潜時を得るために浅い吸入麻酔は有用であり, 熱刺激装置の特性を考慮する必要があることを示した。

短報

片山健太郎・古野亜紀・宮本沙也加・中村美有紀・小鹿　泉・
新海雄介・秋山耕陽・辻　岳人・国枝哲夫
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*Koa*および*Eh*は, ともに第15番染色体上のほぼ同じ領域の逆位に起因するマウス突然変異であり, その表現型には耳介の被毛と頭蓋顔面の形成異常等の類似した部分が認められている。そのため両突然変異個体の表現型は逆位により生じた同一の遺伝子の変異に起因している可能性が考えられた。連鎖解析により組換え抑制領域を特定することで逆位領域を推定したところ, それぞれの逆位領域は異なっていることが判明した。そのため*Koa*および*Eh*の表現型は異なる遺伝子によると推測された。

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ウサギの糞便に排泄したばかりの未芽胞化（unsporulated）*Eimeria piriformis*オーシストを各濃度（1%, 2.5%, 5%および10%）の各クロム化合物（$K_2Cr_2O_7$, CrO_3, $Cr(NO_3)_3$, K_2CrO_4）に処理した後, その芽胞化（sporulation）の程度を観察した。1から10%の$K_2Cr_2O_7$で処理したオーシストは28時間でほとんどのものが芽胞化した。しかし, 2.5%のCrO_3及び$Cr(NO_3)_3$で処理したオーシストが完全芽胞化するのに約60時間を要した。また, 蒸留水, 1%及び10% K_2CrO_4で処理したオーシストの完全芽胞化には, それぞれ216, 156及び96時間を要した。よって*E. piriformis*オーシストを芽胞化させるのに$K_2Cr_2O_7$が最適であることが明らかである。