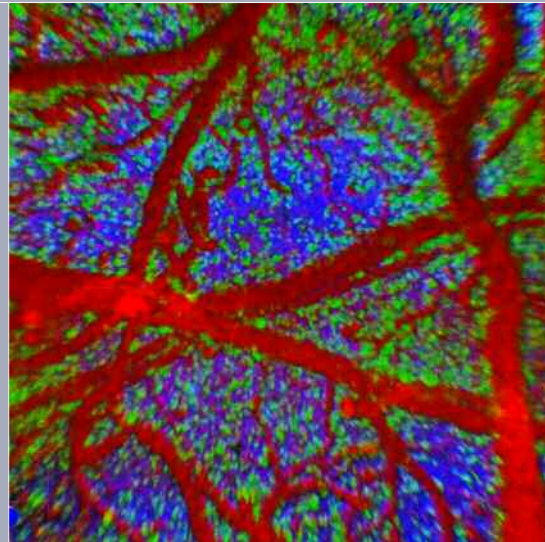


Molecular and Integrative Neurosciences

Viral meningitis in real time. Meningitis is a disease in humans caused by a variety of viruses and bacteria. During the disease, the lining of the brain (or meninges) becomes inundated with inflammatory cells, which induce seizures and profound neurologic dysfunction. In order to gain novel insights into the dynamics of meningitis, an approach was established to visualize the disease in real time through a thinned skull in virus-infected rodents. The image is a 3-dimensional projection of a movie frame captured by using a 2-photon microscope during the peak of meningitis



induced by lymphocytic choriomeningitis virus (LCMV). The area of the projection, just below the surface of the skull (blue), shows profound infiltration of the meningeal space by LCMV-specific cytotoxic lymphocytes (green). The lymphocytes are absolutely required for the seizures that occur during meningitis and induce considerable leakage (or breakdown) in the dense network of meningeal vasculature (red). Work done in the laboratory of Dorian McGavern, Ph.D., associate professor.



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Chairman's Overview

The year 2007 was a successful one for scientists in the Molecular and Integrative Neurosciences Department. Major breakthroughs occurred, and long-term projects proceeded with good focus and urgency.

Highly successful principal investigators such as Bert Weiss, George Siggins, Donna Gruol, and Pietro Sanna, who maintain the highest competence and a critical mass, continue research on substance abuse and addiction. Investigations of arenaviruses by Michael Buchmeier and Juan Carlos de la Torre and their coworkers also continue successfully, as do the studies on brain determinants of energy metabolism by Bruno Conti, Manuel Sánchez-Alavez, and Justin Tabarean.

Cindy Ehlers has long been the authority on substance abuse and obesity in Native Americans, whose share of these disorders is disproportionately high. She has been adding to her epidemiologic studies an important set of findings on genetic markers that explain some of the risk factors that underlie the high prevalence of alcoholism, obesity, and drug abuse. She and her colleagues speculated that because of the history of the Native Americans, after a foraging lifestyle, a selective pressure was placed on retaining genes that favor storing fat for worse times. These scientists went on to postulate that the thrifty genes that promote eating are also the ones

that promote compulsive drug taking and thus may contribute to the drug abuse. In early 2007, Dr. Ehlers and her group showed a link between the obesity and drug abuse and identified several genetic loci on 4 different chromosomes. This extremely important result is a consequence of understanding the spectrum of diseases and will be useful in designing therapies to target processes common to excessive food ingestion and drug abuse.

Dorian McGavern and Michael Oldstone have solved an important puzzle in persistent viral infections, which are a major chronic health problem. It is theorized that viruses have evolved diverse strategies to avoid immunosurveillance and establish persistence throughout the body. Within the brain, viruses hide so cleverly that T cells often have a difficult time even detecting the infected cells. Thus, it was thought that the only way to directly purge persistent viruses was to use antiviral drugs that interfered with steps of viral replication. However, Drs. McGavern and Oldstone recently showed that surveillance by endogenous T cells can be improved by blocking the cytokine IL-10, a host-derived factor that acts as an immunosuppressor. Once IL-10 is removed, T cells can clear a persistent viral infection effectively. These findings are of great theoretical and therapeutic significance. We know that IL-10 can be temporarily neutralized, and several immunotherapies in which antibodies to cytokines neutralize the cytokines are already in use in clinical practice. The breakthrough finding of Drs. McGavern and Oldstone strongly suggests that therapies that block IL-10 and enable T cells to clear viral infections fully and prohibit the establishment of persistence are now possible. From a public health standpoint, persistence is a ticking time bomb, because the viruses can be activated at any time, by a variety of stimuli, to cause undesirable signs and symptoms. Thus, developing highly efficacious therapeutic strategies to thwart viral replication during states of persistence is important.

INVESTIGATORS' REPORTS

HLA-Restricted Epitope Discovery From Pathogenic Arenaviruses

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Arenaviruses are rodent-borne pathogens that cause significant morbidity and mortality in humans. Pathogenic arenaviruses include Lassa, lymphocytic choriomeningitis (LCMV), Junin, Machupo, Guanarito, and Whitewater Arroyo viruses. In human infections with the Old World arenaviruses LCMV or Lassa virus, cellular immunity is thought to play a primary role in viral clearance and protective immunity. Therefore, sensitive reagents are needed to measure the cell-mediated immune response that occurs in humans after infection or in response to possible vaccines. Identification of HLA-restricted epitopes is required to develop assays that can be used to determine the quality of immune responses, define correlates of protection and immunopathologic changes, and ultimately guide the selection of candidate vaccines.

To identify human CD8⁺ T-cell epitopes from pathogenic arenaviruses, we use bioinformatic predictions to identify candidate epitopes, *in vitro* MHC-binding assays and *in vivo* immunogenicity studies in HLA transgenic mice to validate epitopes, and vaccination studies to evaluate whether epitopes protect against viral challenge. During the past year, we used this approach to identify the first human CD8⁺ T-cell epitopes from LCMV. Immunization of HLA-A2 transgenic mice with one of these epitopes, GPC₄₄₇₋₄₅₅, led to significant reductions in viral titer after challenge with LCMV and protected animals from lethal disease.

We also addressed whether HLA-restricted epitopes that are cross-reactive among the pathogenic arenaviruses could be identified for the purpose of developing an epitope-based vaccine that would protect against multiple arenaviruses. We identified a panel of HLA-A*0201-restricted peptides derived from the same region of the GPC gene of Lassa virus (GPC₄₄₁₋₄₄₉), LCMV (GPC₄₄₇₋₄₅₅), Junin virus (GPC₄₂₉₋₄₃₇), Machupo

virus (GPC₄₄₄₋₄₅₂), Guanarito virus (GPC₄₂₇₋₄₃₅), and Whitewater Arroyo virus (GPC₄₂₈₋₄₃₆) that had high-affinity binding to HLA-A*0201 and were recognized by CD8⁺ T cells in a cross-reactive manner after LCMV infection or peptide immunization of HLA-A*0201 transgenic mice. Immunization of HLA-A*0201 mice with the Old World peptides from Lassa virus or LCMV induced high-avidity CD8⁺ T cells that killed syngeneic target cells pulsed with the epitope of either the Lassa virus or LCMV *in vivo* and provided significant protection against viral challenge with LCMV. Our results indicate that HLA-restricted, cross-reactive epitopes exist among diverse arenaviruses and that individual epitopes can be used as effective vaccine determinants for multiple pathogenic arenaviruses.

Coronavirus Supramolecular Structure

B.W. Neuman, R.J. Burrer, J.P.C. Ting, J. Klaus, B.D. Adair,
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Coronaviruses are an important family of human and veterinary pathogens that cause a wide range of diseases. The emergence of the coronavirus that causes severe acute respiratory syndrome highlights a need for structural information on coronavirus proteins and effective antiviral treatments.

Coronaviruses derive their name from the protruding transmembrane spike glycoproteins, which are seated in the viral membrane via interactions with the 3-pass transmembrane matrix glycoprotein. A core containing nucleoprotein and the single-stranded RNA genome of approximately 30 kb is incorporated into virions at membranes of the endoplasmic reticulum–Golgi complex intermediate compartment in a process mediated by interactions between the nucleoprotein and the matrix glycoprotein. We used electron cryomicroscopy and image analysis to examine the supramolecular structure of coronaviruses.

We found that both coronavirus particles and virus-like particles containing only a selected set of viral proteins are enveloped, pleomorphic, and about 80–100 nm in diameter. The oblong shape of the virions appears to be specified by the presence of the viral genomic RNA; particles lacking full-length viral genome are

generally spherical. The homotrimeric surface spikes consist of a globular head supported by a slender stalk. A layer of density directly apposed to the inner bilayer leaflet and tightly packed intramembrane densities are ascribed to molecules of the matrix protein. Punctate densities of about 5 nm are present at the underside of the viral membrane; in purified viral ribonucleoprotein preparations, these densities are ascribed to complexes of nucleoprotein and RNA. Virions appear to derive structural integrity from an interwoven architecture including overlapping 2-dimensional lattices of spike and nucleoprotein molecules appended to the viral membrane. Currently, we are using structural analysis of protein-protein interactions to elucidate the mechanisms of particle formation.

Antisense Antivirals for Coronaviruses and Arenaviruses

R.J. Burrer, B.W. Neuman, J.P.C. Ting, J. Klaus, M.J. Buchmeier

The recent emergence of novel pathogenic human and animal coronaviruses has highlighted the need for antiviral therapies that are effective against a spectrum of these viruses. We have used several strains of murine hepatitis virus in cell culture and in vivo in mouse models to investigate the antiviral characteristics of peptide-conjugated antisense phosphorodiamidate morpholino oligomers (P-PMOs). We tested 10 P-PMOs directed against various target sites in the viral genome in cell cultures. One of these, 5TERM, which was complementary to the 5' terminus of the genomic RNA, was effective against 6 strains of mouse hepatitis virus. Further studies were carried out with various arginine-rich peptides conjugated to 5TERM to evaluate efficacy and toxicity and to select candidates for in vivo testing.

In uninfected mice, prolonged P-PMO treatment did not result in weight loss or detectable histopathologic changes. Treatment with 5TERM reduced viral titers in target organs and protected mice against virus-induced tissue damage. Prophylactic treatment with this P-PMO decreased the amount of weight loss associated with infection under most experimental conditions. Treatment also prolonged survival in 2 lethal challenge models. In some experiments, high-dose viral inoculation followed by delayed treatment with 5TERM was not protective and increased morbidity in the treated group,

suggesting that P-PMOs may cause toxic effects in infected mice that were not apparent in the uninfected animals. However, the strong antiviral effect suggests that with further development, P-PMOs may provide an effective therapeutic approach against a broad range of coronavirus infections.

Vaccination for Severe Acute Respiratory Syndrome–Associated Coronavirus

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In an effort to develop a multiepitope vaccine against severe acute respiratory syndrome–associated coronavirus (SARS-CoV), we are collaborating with investigators at the La Jolla Institute of Allergy and Immunology. We are attempting to identify epitopes within the 4 structural proteins (nucleoprotein, matrix, envelope, and spike) of the virus that can be presented on human MHC class I molecules. Although SARS-CoV contains at least 14 open reading frames, the 4 structural proteins have the highest level of expression in cells infected with the virus. Hence, in a natural infection, these 4 proteins are the ones most likely to be processed and presented on MHC class I molecules.

On the basis of a predictive algorithm, several potential epitopes within these 4 proteins have been identified. These predicted epitopes have been synthesized as 9mer peptides and have been tested for in vitro binding affinities to MHC class I molecules of the A1, A2, A3, A24, B7, and B44 supertypes. This process has yielded a pool of peptides that must be further tested for their ability to elicit a CD8⁺ T-cell response in vivo.

To determine whether these candidate peptides are immunogenic in vivo, we will use IFN- γ enzyme-linked immunospot assays. Initially, HLA transgenic mice will be immunized with pools of candidate peptides. After 2 weeks, CD8⁺ T cells will be isolated from the spleens of the mice and exposed to antigen-presenting cells that have been infected with recombinant vaccinia virus that expresses 1 of the 4 SARS-CoV

structural proteins. If a SARS-CoV protein can be processed such that a predicted epitope within that protein can be loaded onto an MHC class I molecule, an antigen-presenting cell expressing that particular SARS-CoV protein should be able to stimulate a memory response in CD8⁺ T cells that previously encountered that epitope. The presence or absence of a memory response can then be determined by monitoring for the secretion of IFN- γ . Construction of recombinant vaccinia viruses that express the 4 structural proteins of SARS-CoV has been completed and immunization of HLA transgenic mice with the various candidate peptides is under way.

Pathogenesis of Encephalitis and Demyelination

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We have examined the outcome of viral encephalomyelitis caused by 3 different viruses, mouse hepatitis virus A59, Theiler's encephalomyelitis virus, and coxsackievirus B3, in mice with autoantibodies to a CNS-specific antigen, myelin oligodendrocyte glycoprotein, in which no clinical disease usually develops. Morbidity and mortality associated with the acute viral CNS disease was augmented by the presence of the autoantibodies in all 3 viral infections. Transfer of serum containing the autoantibodies at the time of infection with mouse hepatitis virus was sufficient to reproduce the exacerbated disease. The presence of the autoantibodies caused increased infiltration of mononuclear cells into the brain.

In mice with the CNS-specific autoantibodies, infection with mouse hepatitis virus led to marked augmentation of early demyelination in the brain and spinal cord. The antibody-mediated exacerbation was independent of the complement system but required expression of Fc receptors; the exacerbation occurred in mice deficient in C3 but not in mice deficient in the Fc receptor. Our results suggest that infections can lead to much more profound immunopathologic effects when an otherwise latent autoimmune condition is present.

Structure and Function of the Arenavirus Signal Peptide

A.A. Saunders, B.W. Neuman, J.P.C. Ting, M.J. Buchmeier

The stable signal peptide of the surface glycoprotein precursor in lymphocytic choriomeningitis virus has several unique characteristics. The peptide is unusually long at 58 amino acids, it contains 2 hydrophobic domains, and its sequence is highly conserved among both Old and New World arenaviruses. To better understand the functions of the peptide, we created a panel of point and deletion mutants to target the highly conserved elements within the peptide. Using *trans*-complementation, we were also able to confirm critical residues required for separate functions of the stable signaling peptide. With these approaches, we were able to resolve functional domains of the peptide.

In characterizing our mutants, we discovered that the stable signaling peptide is involved in several distinct functions within the viral life cycle, beyond translocation of the viral surface glycoprotein precursor into the lumen of the endoplasmic reticulum. The peptide is required for efficient glycoprotein expression, post-translational maturation cleavage of glycoproteins 1 and 2 by SKI-1/S1P protease, glycoprotein transport to the cell-surface plasma membrane, formation of infectious virus particles, and acid pH-dependent glycoprotein-mediated cell fusion.

PUBLICATIONS

Botten, J., Whitton, J.L., Barrowman, P., Sidney, J., Whitmire, J.K., Alexander, J., Ting, J.P., Bui, H.H., Sette, A., Buchmeier, M.J. HLA-A2-restricted protection against lethal lymphocytic choriomeningitis. *J. Virol.* 81:2307, 2007.

Buchmeier, M.J., de la Torre, J., Peters, C.J. Arenaviruses: the viruses and their replication. *In: Fields Virology*, 5th ed. Knipe, D.M., Howley, P.M. (Eds.-in-Chief). Lippincott Williams & Wilkins, Philadelphia, 2007, p. 1792.

Bui, H.H., Botten, J., Fussedner, N., Pasquetto, V., Mothe, B., Buchmeier, M.J., Sette, A. Protein sequence database for pathogenic arenaviruses. *Immunome Res.* 3:1, 2007.

Burrer, R., Buchmeier, M.J., Wolfe, T., Ting, J.P.C., Feuer, R., Iglesias, A., von Herrath, M.G. Exacerbated pathology of viral encephalitis in mice with central nervous system-specific autoantibodies. *Am. J. Pathol.* 170:557, 2007.

Burrer, R., Neuman, B.W., Ting, J.P.C., Stein, D.A., Moulton, H.M., Iversen, P.L., Kuhn, P., Buchmeier, M.J. Antiviral effects of antisense morpholino oligomers in murine coronavirus infection models. *J. Virol.* 81:5637, 2007.

Burrer, R., von Herrath, M.G., Wolfe, T., Rempel, J.D., Iglesias, A., Buchmeier, M.J. Autoantibodies exacerbate the severity of MHV-induced encephalitis. *Adv. Exp. Med. Biol.* 581:399, 2006.

Joseph, J.S., Saikatendu, K.S., Subramanian, V., Neuman, B.W., Buchmeier, M.J., Stevens, R.C., Kuhn, P. Crystal structure of a monomeric form of severe acute respiratory syndrome coronavirus endonuclease nsp15 suggests a role for hexamerization as an allosteric switch. *J. Virol.* 81:6700, 2007.

Kotturi, M.F., Peters, B., Buendia-Laysa, F., Jr., Sidney, J., Oseroff, C., Botten, J., Grey H., Buchmeier, M.J., Sette, A. The CD8⁺ T-cell response to lymphocytic choriomeningitis virus involves the L antigen: uncovering new tricks for an old virus. *J. Virol.* 81:4928, 2007.

Mothe, B.R., Stewart, B.S., Oseroff, C., Bui, H.H., Stogiera, S., Garcia, Z., Dow, C., Rodriguez-Carreno, M.P., Kotturi, M., Pasquetto, V., Botten, J., Crotty, S., Janssen, E., Buchmeier, M.J., Sette, A. Chronic lymphocytic choriomeningitis virus infection actively down-regulates CD4⁺ T cell responses directed against a broad range of epitopes. *J. Immunol.* 179:1058, 2007.

Neuman, B.W., Adair, B.D., Yoshioka, C., Quispe, J.D., Milligan, R.A., Yeager, M., Buchmeier, M.J. Ultrastructure of SARS-CoV, FIPV, and MHV revealed by electron cryomicroscopy. *Adv. Exp. Med. Biol.* 581:181, 2006.

Neuman, B.W., Stein, D.A., Kroeker, A.D., Moulton, H.M., Bestwick, R.K., Iversen, P.L., Buchmeier, M.J. Inhibition and escape of SARS-CoV treated with antisense morpholino oligomers. *Adv. Exp. Med. Biol.* 581:567, 2006.

Reignier, T., Oldenburg, J., Noble, B., Lamb, E., Romanowski, V., Buchmeier, M.J., Cannon, P.M. Receptor use by pathogenic arenaviruses. *Virology* 353:111, 2006.

Saikatendu, K.S., Joseph, J.S., Subramanian, V., Neuman, B.W., Buchmeier, M.J., Stevens, R.C., Kuhn, P. Ribonucleocapsid formation of severe acute respiratory syndrome coronavirus through molecular action of the N-terminal domain of N protein. *J. Virol.* 81:3913, 2007.

Saunders, A.S., Ting, J.P.C., Meisner, J., Neuman, B.W., Perez, M., de la Torre, J.C., Buchmeier, M.J. Mapping the landscape of the lymphocytic choriomeningitis virus stable signal peptide reveals novel functional domains. *J. Virol.* 81:5649, 2007.

Chronic Virus-Host Interaction in the CNS

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The brain is a unique organ, not only functionally but also in terms of host response to events such as infection. We study processes in which this response leads to brain dysfunction; we have mostly focused on a degenerative and dementing condition that arises after a known stimulus, infection with HIV. Using infection of rhesus monkeys with simian immunodeficiency virus (SIV) as a model of neuroAIDS, we are studying the virology, immunology, pathology, and neurobiology of the resulting CNS disease.

We have defined the different stages of SIV disease in the CNS in terms of interactions between the virus and the immune system, pathologic changes in CNS function, and molecular mechanisms. In particular, we characterized both the innate response of the brain's intrinsic defense system and the development of the adaptive response by the immune system, with its characteristics in the brain.

Interestingly, during the chronic phase of SIV infection, although only a low amount of virus is present in

the brain, SIV-specific cytotoxic T lymphocytes are present in relatively high numbers. Furthermore, these lymphocytes in the brain have specificities not found in cytotoxic T lymphocytes in the rest of the body. The maintenance of these cells in the brain is linked to the upregulation of IL-15 in the brain, creating a unique environment in which cells are exposed to IL-15 in the absence of IL-2, likely leading to their enrichment and persistence.

Infected macrophages in the brain drive the CNS abnormalities that occur in HIV infection. Although the accumulation of brain macrophages is the best correlate of CNS dysfunction, the mechanisms by which blood monocytes enter the brain and persist as brain macrophages is still unknown. We have found that osteopontin plays an important role in this process. Although chemokines can increase monocyte entry, osteopontin keeps monocytes that migrate into organs such as the brain within the tissue, leading to accumulation of the cells. Furthermore, osteopontin is antiapoptotic for blood monocytes, increasing their survival. We have found that osteopontin is elevated in both the blood and brain of monkeys with CNS disease. Currently, we are examining its role in humans with HIV infection.

Understanding the mechanisms of dysfunction in the brain will define the cause and development of CNS HIV infection and, potentially, other CNS disorders and lead to preventative or therapeutic strategies.

PUBLICATIONS

Burdo, T.H., Wood, M.R., Fox, H.S. Osteopontin prevents monocyte recirculation and apoptosis. *J. Leukoc. Biol.* 81:1504, 2007.

Chatterji, U., Bobardt, M.D., Gaskill, P.J., Sheeter, D., Fox, H.S., Galloway, P.A. Trim5 α accelerates degradation of cytosolic capsid associated with productive HIV-1 entry. *J. Biol. Chem.* 281:37025, 2006.

Marcondes, M.C., Burdo, T.H., Sopper, S., Huitrón-Reséndiz, S., Lanigan, C.M., Watry, D.D., Flynn, C.F., Zandonatti, M.A., Fox, H.S. Enrichment and persistence of virus-specific CTL in the brain of simian immunodeficiency virus-infected monkeys is associated with a unique cytokine environment. *J. Immunol.* 178:5812, 2007.

Marcondes, M.C., Penedo, M.C., Lanigan, C., Hall, D., Watry, D.D., Zandonatti, M., Fox, H.S. Simian immunodeficiency virus-induced CD4⁺ T cell deficits in cytokine secretion profile are dependent on monkey origin. *Viral Immunol.* 19:679, 2006.

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The Viral-Immunobiology Laboratory encompasses the programs of 4 faculty members: Juan Carlos de la Torre, Stefan Kunz, Dorian McGavern, and Michael B.A. Oldstone. Each program is independent, but the interactions between the researchers and the use of different technologies provide an intellectual sum greater than any single part. Our studies of both viral and transmissible spongiform encephalopathies (e.g., prion diseases, scrapie) include basic analysis of the mechanisms by which viruses persist, escape immune recognition, and cause disease. Integral parts of the programs are understanding how viruses infect cells; defining the cellular receptors used by viruses; and mapping the trafficking of viruses into and in the cells and the subsequent viral uncoating, replication, assembly, exit, and spread. Because the immune system has evolved to recognize, attack, and remove these foreign substances, we evaluate the immune response against viruses, probe how viruses subvert this response to provide a selective advantage for their survival, and study how the host can correct this subversion to allow termination of viral persistence.

Other interests include dissecting how viruses and immune cells traffic to the brain and interact there; how viruses are cleared from the brain, and how viruses alter the differentiation processes of cells they persistently infect, thereby disturbing homeostasis and causing disease. We are also investigating how viruses induce autoimmune disease or induce immunosuppression, and we are designing therapies to control viral infections. Because different viruses have different lifestyles, we focus on 3 RNA negative-stranded viruses: Borna disease virus, lymphocytic choriomeningitis virus, and measles virus. We also investigate the mechanism by

which infectious agents cause transmissible spongiform encephalopathies. In addition, recently in collaboration with H. Rosen, Department of Immunology, we are studying the immunobiology of influenza virus infection.

Cure of Persistent Viral Infection by Blockade of IL-10

D.G. Brooks, M.B.A. Oldstone

IL-10 is elevated in persistent viral infections caused by lymphocytic choriomeningitis virus in mice, the natural host of this virus, and is increased in persistent infections caused by HIV and hepatitis B and C viruses in humans. We found that in vivo antibody blockade of the IL-10 receptor prevented persistence of lymphocytic choriomeningitis virus when given early in infection. More important, when antibody was given late during the course of persistence, when T lymphocytes are no longer functioning (are exhausted), function of T cells is resurrected. That is, proliferation and secretion of the antiviral cytokines IFN- γ and TNF- α are restored. Once function is returned, T cells are able to control infection and clear the virus.

Suppression of Adaptive and Innate Immune Responses to Measles Virus and Lymphocytic Choriomeningitis Virus via Interactions Between the Virus and Dendritic Cells

B. Hahm, E. Zuniga, L. Liou, M.B.A. Oldstone

ADAPTIVE IMMUNITY

To dissect the molecular basis of interactions between measles virus and dendritic cells and the resultant immunosuppression, we developed transgenic mice that express SLAM, a receptor for measles virus, solely on dendritic cells. Dendritic cells from the transgenic mice expressed the human SLAM protein and were susceptible to measles virus infection in vitro and in vivo. Measles virus infection inhib-

ited the development of dendritic cells from hematopoietic bone marrow stem cells in cultures supplemented with either granulocyte-monocyte colony-stimulating factor or the ligand for Flt3, another growth factor. Suppression of dendritic cell development caused by measles virus infection was not reproduced when transgenic bone marrow cells were deficient in the receptor for type I interferon, indicating that virus-induced type I interferons prevented dendritic cell precursors from differentiating into dendritic cells. Direct treatment of cultures with recombinant IFN- β recapitulated the effect of measles virus infection by inhibiting the development of dendritic cells.

In contrast to the usual finding that interferon signaling activates both signal transducer and activator of transcription (STAT) 1 and STAT2, type I interferons induced by measles virus required expression of STAT2 but not that of STAT1, STAT4, or STAT6 to inhibit the development of dendritic cells. Expression of STAT2 induced by measles virus maintained its phosphorylation status in normal or STAT1-deficient cells, activating gene transcription dependent on the interferon-stimulated response elements. In the absence of STAT2, treatment with measles virus stimulated differentiation of dendritic cells expressing CD11c, MHC-II, CD40, B7-1, or B7-2. Measles virus or recombinant IFN- β markedly increased the total number of cells deficient in STAT2. With a loss of STAT2 protein, type I interferons became mitogenic, leading to rapid cell proliferation. Pro-proliferative signaling mediated by type I interferons occurred in the absence of either STAT2 alone or both STAT1 and STAT2, suggesting that type I interferons trigger an unknown signaling cascade but not canonical STAT1/2 signaling.

On the other hand, in the absence of STAT1, recombinant IFN- β had contradictory dual activity; it induced proliferation and differentiation of dendritic cells at low concentrations but suppressed dendritic cells at higher concentrations. Low doses of measles virus suppressed development of dendritic cells lacking STAT1, indicating that type I interferons induced by the virus trigger STAT2-selective signaling to override the positive signaling mediated by the interferons in the absence of STAT1.

Thus, STAT2 is a "molecular switch" used by type I interferons induced by measles virus in dendritic cell precursors that control the development of dendritic cells (enhancement vs suppression). Measles virus-mediated activation of STAT2 overcomes the stimulatory activity

of type I interferons on dendritic cells, resulting in suppression of the cells in measles. Because STAT1-independent, STAT2-dependent signaling is a unique interferon signaling that leads to inhibition of the development of dendritic cells, targeting STAT2-selective signaling might increase the efficacy of interferon therapy or be useful in the development of novel drugs against diseases caused by immunosuppression or viral persistence.

INNATE IMMUNITY

As a cellular component for detecting microbial pathogens, Toll-like receptors (TLR)s on dendritic cells are effective molecular sensors that recognize various molecular signatures. We found that lymphocytic choriomeningitis virus (LCMV) infection of its natural host, mice, interferes with TLR7 and TLR9, but not TLR4, responses on plasmacytoid and conventional dendritic cells, inhibiting the production of type I interferons, IL-12, and TNF- α both in vivo and in vitro. These data reveal, for the first time, that the ability of the innate immune system to respond to in vivo TLR stimulation is differentially modulated during a viral infection.

Suppression of the response of dendritic cells to TLR9 was independent of viral replication within dendritic cells; it was a consequence of the infectious environment. The in vivo response to TLR9 challenge of LCMV-infected mice deficient in the genes for the receptors for type I interferons or STAT2 was similar to the response of uninfected control mice, although with a delay. This finding was in contrast to the responses of wild-type or STAT1-deficient mice when the mice were infected with LCMV. Moreover, treatment with IFN- β also inhibited TLR9 responses, indicating that type I interferons secreted during LCMV infection acted on dendritic cells through a STAT2-dependent, STAT1-independent pathway. Importantly, LCMV strain CI 13, which causes a persistent infection, induced a long-term suppression of the TLR9 response on dendritic cells. This suppression prevented the host from mounting an effective innate immune response to a secondary opportunistic pathogen such as murine cytomegalovirus, which is normally recognized by TLR9. Consequently, natural killer cells from mice infected with LCMV CI 13 had reduced activation, compromised secretion of IFN- γ , and decreased cytotoxic activity after the mice were infected with murine cytomegalovirus.

Measles virus also influenced the TLR system of dendritic cells. Infection with the virus affects TLRs and impairs the ability of dendritic cells to produce IL-12

in response to stimulation with lipopolysaccharide, a ligand for TLR4. However, IL-12 inhibition did not occur when measles virus–infected dendritic cells were stimulated with ligands for TLR2, TLR3, TLR7, and TLR9. Synthesis of other cytokines such as IL-6 and TNF- α was not suppressed in any TLR stimulation, including TLR4, suggesting that measles virus inhibits IL-12 production specifically. This inhibition of IL-12 in response to lipopolysaccharide stimulation of dendritic cells infected with measles virus was dominant over other TLR stimulations; the IL-12 suppression still occurred after treatment with lipopolysaccharide plus CpG, poly (I:C), loxoribine, or peptidoglycan. This result suggests that measles virus makes infected individuals vulnerable to secondary microbes, which are normally sensitive to TLR4/IL-12–mediated immune responses of infected dendritic cells. Further, specific targeting of TLR4/IL-12 by measles virus likely explains a biased type 2 helper T cell phenotype in patients with measles, because IL-12 plays a major role in selecting for immune responses that involve type 1 helper T cells.

Pathogenesis of Chronic Wasting (Prion) Disease of Deer and Elk

M.J. Trifilo, C. Teng, M.B.A. Oldstone

Chronic wasting disease is both a major economic and a public health concern. To understand the host genetic susceptibility, spread of infectious material, and pathogenesis, we developed transgenic mice with our collaborator B. Chesebro, Rocky Mountain Laboratories, Hamilton, Montana. Because epidemiologic reports indicated that allelic variation in deer prion protein (PrP) correlated with susceptibility, we developed mice that expressed either a glycine or a serine in residue 96 of deer PrP. After infection with the agent that causes deer scrapie, transgenic mice with glycine at position 96 were significantly more susceptible than those with a serine at that position.

Using transgenic mice with glycine at position 96, we found that oral inoculation with the agent that causes deer scrapie resulted in disease. At 200 days after inoculation, when the mice were clinically healthy, we detected PrPres, an abnormally folded PrP, in the dorsal surface of the tongue, primarily in serous and mucosa glands; in the intestine; in the spleen; and in the olfactory bulb and brain stem. We did not detect any PrPres

in cerebellum, cerebral cortex, or hippocampus. When the mice became clinically ill 150 days later, we detected PrPres throughout the brain, which also had massive hyperreactive astrocytosis, neuronal dropout, and florid plaques.

Thus, a model has been developed that should provide leads for determining when samples from deer with chronic wasting disease contain infectious material, what tissues contain infectious material, and how much infectious material the tissues contain. The model should also provide a better understanding of the spread and pathogenesis of chronic wasting disease.

PUBLICATIONS

Brooks, D.G., Trifilo, M.J., Edelmann, K.H., Teyton, L., McGavern, D.B., Oldstone, M.B.A. Interleukin-10 determines viral clearance or persistence in vivo. *Nat. Med.* 12:1301, 2006.

Go, E.P., Wikoff, W., Shen, Z., O'Maille, G., Morita, H., Conrads, T.P., Nordstrom, A., Trauger, S.A., Uritboonthai, W., Lucas, D.A., Chan, K.C., Veenstra, T.D., Lewicki, H., Oldstone, M.B., Schneemann, A., Siuzdak, G. Mass spectrometry reveals specific and global molecular transformations during viral infection. *J. Proteome Res.* 5:2405, 2006.

Hahn, B., Cho, J.-H., Oldstone, M.B.A. Measles virus-dendritic cell interaction via SLAM inhibits innate immunity: selective signaling through TLR4 but not other TLRs mediates suppression of IL-12 synthesis. *Virology* 358:251, 2007.

Homann, D., Lewicki, H., Brooks, D., Eberlein, J., Mallet-Designe, V., Teyton, L., Oldstone, M.B.A. Mapping and restriction of a dominant viral CD4⁺ T cell core epitope by both MHC class I and MHC class II. *Virology* 363:113, 2007.

Kunz, S., Rojek, J.M., Roberts, A.J., McGavern, D.B., Oldstone, M.B.A., de la Torre, J.C. Altered central nervous system gene expression caused by congenitally acquired persistent infection with lymphocytic choriomeningitis virus. *J. Virol.* 80:9082, 2006.

Lauterbach, H., Zuniga, E.I., Truong, P., Oldstone, M.B.A., McGavern, D.B. Adoptive immunotherapy induces CNS dendritic cell recruitment and antigen presentation during clearance of a persistent viral infection. *J. Exp. Med.* 203:1963, 2006.

Meade-White, K., Race, B., Trifilo, M., Bossers, A., Favara, C., Lacasse, R., Miller, M., Williams, E., Oldstone, M., Race, R., Chesebro, B. Resistance to chronic wasting disease in transgenic mice expressing a naturally occurring allelic variant of deer prion protein. *J. Virol.* 81:4533, 2007.

Oldstone, M.B.A. A suspenseful game of 'hide and seek' between virus and host. *Nat. Immunol.* 8:325, 2007.

Trifilo, M.J., Hahn, B., Zuniga, E.I., Edelmann, K.H., Oldstone, M.B.A. Dendritic cell inhibition: memoirs from immunosuppressive viruses. *J. Infect. Dis.* 194(Suppl. 1):S3, 2006.

Trifilo, M.J., Ying, G., Teng, C., Oldstone, M.B.A. Chronic wasting disease of deer and elk in transgenic mice: oral transmission and pathobiology. *Virology* 365:136, 2007.

Zuniga, E.I., Hahn, B., Oldstone, M.B.A. Type I interferon during viral infections: multiple triggers for a multifunctional mediator. *Curr. Top. Microbiol. Immunol.* 316:337, 2007.

Arenavirus Molecular and Cell Biology: Implications for Novel Antiviral Therapies

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Arenaviruses are important both as model systems for studies of acute and persistent viral infections and as human pathogens, including Lassa virus and other agents that cause hemorrhagic fever. Moreover, evidence indicates that the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) is a neglected human pathogen of clinical importance for which no licensed vaccines are available; current therapy is limited to the use of ribavirin, which is only partially effective and is associated with severe side effects.

Arenaviruses are enveloped viruses with a bisegmented negative-stranded RNA genome. Each genomic RNA segment, S and L, uses an ambisense coding strategy to direct the synthesis of 2 polypeptides. The S RNA encodes the viral glycoprotein and the nucleoprotein, whereas the L RNA encodes the viral RNA-dependent RNA polymerase and the small RING finger protein Z. We have developed a reverse genetics system for LCMV that enables us to re-create all the steps of the virus life cycle from cloned cDNA. This system provides a novel and powerful approach for elucidating the role of viral polypeptides and *cis*-acting sequences in the control of arenavirus RNA replication, gene expression, and assembly and for identifying and functionally characterizing virus-cell protein interactions that contribute to arenavirus pathogenesis.

Using this system we have characterized the arenavirus genome promoter and polymerase and discovered that the virus small RING finger Z protein is the driving force of arenavirus budding. On the basis of these results, we are developing novel strategies to combat pathogenic arenaviruses by inhibiting 2 essential steps of the arenavirus life cycle: RNA synthesis mediated by the viral polymerase and viral budding.

LCMV is a Rosetta stone in viral immunology and pathogenesis. We can now generate recombinant LCMVs that have predetermined specific mutations within their genomes and analyze their phenotypic expression *in vivo*, a novel and powerful approach for elucidating the molecular mechanisms that underlie arenavirus-host interactions, including the mechanisms that facilitate arenavirus persistence and associated disorders.

PUBLICATIONS

Kunz, S., Rojek, J.M., Roberts, A.J., McGavern, D.B., Oldstone, M.B.A., de la Torre, J.C. Altered central nervous system gene expression caused by congenitally acquired persistent infection with lymphocytic choriomeningitis virus. *J. Virol.* 80:9082, 2006.

Martínez-Sobrido, L., Zúñiga, E., Rosario, D., García-Sastre, A., de la Torre, J.C. Inhibition of type I interferon response by the nucleoprotein of the prototypic arenavirus lymphocytic choriomeningitis virus. *J. Virol.* 80:9192, 2006.

Saunders, A.A., Ting, J.P.C., Meisner, J., Neuman, B., Perez, M., de la Torre, J.C., Buchmeier, M.J. Mapping the landscape of the lymphocytic choriomeningitis virus stable signal peptide reveals novel functional domains. *J. Virol.* 81:5649, 2007.

Acute and Persistent Viral Infection of the CNS

D.B. McGavern, P. Truong, S. Kang, L. Garidou, H. Lauterbach

We focus on 3 important areas pertaining to viral infection of the CNS: viral pathogenesis during acute and persistent infection, strategies to clear established persistent infections, and development of novel small molecules to antagonize CNS immunopathologic changes. Viral infection of the CNS is a unique challenge to a host because a delicate balance between virus eradication and immunopathologic changes must be maintained. We are using a model pathogen, lymphocytic choriomeningitis virus (LCMV), to mechanistically understand both sides of this balance. We recently showed that through experimental manipulation, we can model infection of almost every major cell population in the CNS (i.e., neurons, astrocytes, oligodendrocytes, meningeal cells, ependymal cells, and choroid plexus) by using LCMV. This diverse assortment of models in combination with the advanced methods available in the LCMV model system enables us to further our understanding of immune interactions in the CNS.

To understand viral pathogenesis during states of acute and persistent infection, we recently used intravital 2-photon microscopy to conduct the first real-time studies to visualize interactions between virus and the immune system in the meninges and layer 1 neocortex. Using this approach, we have acquired 4-dimensional visualizations of fluorescently tagged virus-specific cytotoxic T lymphocytes mediating fatal LCMV-induced meningitis. We have also used this approach to visualize and quantify the real-time breakdown of the blood-brain barrier, microglial activation, and astrocytic calcium flux that occurs in response to infection in the CNS. One of the most important aspects of using this tech-

nique is the ability to dissect viral pathogenesis in real time at the site of injury. We fully anticipate that this approach will rapidly increase our understanding of CNS viral pathogenesis and mechanisms of clearance.

We are also focusing on gaining insights into a remarkable therapeutic approach referred to as immunocytotherapy. This approach relies on administering virus-specific memory T cells to a persistently infected host. Specifically, we exploit a well-established model in which mice are persistently infected from birth or in utero with LCMV. Quite remarkably, a single injection of LCMV-specific memory T cells into LCMV carrier mice results in eradication of the virus from the periphery as well as from CNS neurons without evidence of neuronal injury.

We are defining the precise mechanisms by which adoptively transferred memory T cells purge neurons of a persistent viral infection. We recently found that LCMV-specific T cells arrive in the CNS early after adoptive immunotherapy and recruit dendritic cells that act as antigen-presenting cells. We also discovered that these host-derived dendritic cells are required for successful immunotherapy. Currently, we are determining precisely how dendritic cells facilitate the activities of the transferred memory T cells. This research is particularly intriguing because dendritic cells are not normally found in the resting CNS parenchyma, and immunologic dogma states that memory cytotoxic T lymphocytes (unlike their naive counterparts) should no longer be reliant on antigen-presenting cells.

In conclusion, our long-term objective is to gain fundamental insights into the interrelationships between 2 highly evolved mammalian systems, the CNS and the immune system, particularly in relation to the control of CNS viral infections and the development of immune-mediated neurologic diseases in humans. To this end, we have established collaborations with a cross-disciplinary team of researchers with expertise in metallo-organic chemistry, molecular virology, and intravital 2-photon microscopy to examine the dynamics of CNS immune surveillance and the precise factors that contribute to injurious vs noninjurious outcomes. We hope this knowledge will ultimately lead to interventions to alleviate virus-induced CNS diseases in humans.

PUBLICATIONS

Brooks, D.G., Trifilo, M.J., Edelmann, K.H., Teyton, L., McGavern, D.B., Oldstone, M.B.A. Interleukin-10 determines viral clearance or persistence in vivo. *Nat. Med.* 12:1301, 2006.

Kunz, S., Rojek, J.M., Roberts, A.J., McGavern, D.B., Oldstone, M.B.A., de la Torre, J.C. Altered central nervous system gene expression caused by congenitally acquired persistent infection with lymphocytic choriomeningitis virus. *J. Virol.* 80:9082, 2006.

Tishon, A., Lewicki, H., Andaya, A., McGavern, D., Martin, L., Oldstone, M.B. CD4 T cell control primary measles virus infection of the CNS: regulation is dependent on combined activity with either CD8 T cells or with B cells: CD4, CD8 or B cells alone are ineffective. *Virology* 347:234, 2006.

Arenavirus Entry and Development of Antiviral Drugs Against Human Pathogenic Arenaviruses

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Several arenaviruses cause severe hemorrhagic fevers with high mortality in humans. The most important human pathogenic arenavirus is Lassa virus, which causes more than 300,000 infections and thousands of death annually. In the Americas, the South American viruses Junin, Machupo, and Guanarito have emerged as etiologic agents of severe hemorrhagic fevers. Apart from the humanitarian burden of the viral infections in endemic regions, air travel regularly imports arenavirus hemorrhagic fever into metropolitan areas around the globe, placing local populations at risk.

Our research combines the study of fundamental mechanisms of arenavirus infections with the development of novel antiviral strategies against these devastating diseases. Human pathogenic arenaviruses are highly lethal because once inside the body, they multiply rapidly and overwhelm the host's immune defense, resulting in uncontrolled fatal infection. A promising target for therapeutic intervention is therefore the first step of viral infection. Preventing viral entry would slow down viral spread and provide the host's immune system a window of opportunity to develop an antiviral immune response.

In one part of our program, we are characterizing the cell-surface receptors of pathogenic arenaviruses and investigating the cellular mechanisms of arenavirus entry. The cellular receptor for Lassa virus is α -dystroglycan, a cell-surface receptor for proteins of the extracellular matrix. Recently, we found that Lassa virus recognizes highly conserved glycan structures on α -dystroglycan that are also involved in binding to the extracellular matrix. As a consequence, Lassa virus efficiently competes with proteins of the matrix and perturbs the normal function of α -dystroglycan. Using a combination of biochemical and cell biological techniques, we

are studying the effect of virus binding on α -dystroglycan-mediated signal transduction and cell-matrix adhesion. Upon receptor binding, arenaviruses enter the cell by unknown endocytotic pathways that deliver the viruses to endosomes, where pH-dependent fusion of the viral envelope and the cellular membrane occur. Currently, we are characterizing the endocytotic pathways used by arenaviruses to invade the host cell.

A major goal of our research is the development of novel antiviral drugs that can block these initial steps of infection. Using high-throughput screening assays for compounds in libraries of combinatorial small molecules provided by D.L. Boger, Department of Chemistry, we have identified a number of candidate compounds that specifically block attachment and entry of pathogenic arenaviruses into human cells. We are optimizing, pharmacologically characterizing, and determining the exact mechanism of action of the most promising compounds.

PUBLICATIONS

Rojek, J.M., Spiropoulou, C.F., Campbell, K.P., Kunz, S. Old World and clade C New World arenaviruses mimic the molecular mechanism of receptor recognition used by α -dystroglycans's host-derived ligands. *J. Virol.* 81:5685, 2007.

Viral Pathogenesis and Antiviral Immunity

J.L. Whitton, N. An, N. Benning, C. Cornell, S. Crocker, B. Eam, R. Feuer, R. Frausto, S. Harkins, C. Kembal, F. Liu, J. Whitmire

ANTIVIRAL T CELLS: REGULATION OF QUALITY AND QUANTITY

CD8⁺ T cells play a key role in combating most viral infections, either by killing virus-infected cells or by showering the cells with antiviral cytokines such as IFN- γ . During microbial infection, epitope-specific CD8⁺ T-cell responses usually exist as a hierarchy; responses to some epitopes are much stronger than responses to others. The stronger responses are termed dominant; the weaker, subdominant. The hierarchy is regulated by a poorly understood phenomenon called immunodominance.

We have found that immunodominance depends on expression of IFN- γ . Our current hypothesis is that the immunodominance hierarchy (i.e., the relative abundances of the various epitope-specific T-cell populations) is defined by the rate at which the various epitope-specific cells can initiate production of IFN- γ ;

the fastest cells become the dominant population during the primary immune response. Our most recent data indicate that expression of receptors for IFN- γ on CD8⁺ T cells is tightly regulated and that cells lacking these receptors are at a selective disadvantage. In the absence of this receptor, the frequency of memory cells after viral infection is decreased approximately 100-fold. Therefore, evolution appears to have used IFN- γ to kill two birds with one stone; the cells that are best suited to combat viral infection (i.e., the cells that most rapidly elaborate IFN- γ) are the cells that are preferentially expanded in the host during primary infection and preferentially enter the memory pool. These studies of T-cell regulation are being extended to include CD4⁺ T cells.

In most studies of T-cell function, including ours, synthetic peptides are used to stimulate T-cell responses *in vitro*. We have developed a novel method to identify T cells, and other cell types, that are actively responding to contact with authentic antigen *in vivo*. Using this approach, we showed that the *in vivo* response of CD8⁺ memory T cells to viral infection is explosive. This method not only will be useful for studies of immune responses to infection but also may facilitate a better understanding of autoimmune disease. In our analysis of antigen-specific activation *in vivo*, we also use *in situ* hybridization, and we have confirmed that the *in vivo* CD8⁺ T-cell IFN- γ response to antigen contact is very rapid and is regulated at the transcriptional level.

VIRAL PATHOGENESIS

Coxsackievirus B3 is an important human pathogen that causes a variety of clinical syndromes, including myocarditis and pancreatitis. Myocarditis is remarkably common (about 1 million cases per year in the United States), currently is not treatable, and can lead to dilated cardiomyopathy, which is the most common indicator for heart transplantation in young males. We previously showed the importance of CD4⁺ and CD8⁺ T cells in the control of virus-induced myocarditis and in the related immunopathologic changes.

We are extending our studies of coxsackievirus B3-specific immune responses to ask why this virus does not induce strong CD8⁺ T-cell responses, despite reaching very high concentrations in various tissues. We have prepared several recombinant forms of the virus that express well-characterized CD4⁺ and CD8⁺ epitopes, and we have evaluated the *in vivo* presentation of these epitopes by using epitope-specific T cells as indicators. Our results indicate that coxsackievirus

B3 does a good job of blocking the presentation of CD8⁺ epitopes. What might be the underlying mechanism? We have found that several of the viral proteins target the Golgi complex, inhibiting the function of the complex and leading to its eventual dissolution.

Furthermore, the virus appears to accelerate cellular endocytosis, denuding the cell membrane of MHC class I molecules and thereby making infected cells invisible to CD8⁺ T cells. We postulate that other proteins, such as cytokine receptors, also may be removed from the cell surface, making infected cells untouchable by cytokines, which are key effectors of the host's antiviral response. Recently, we found that blockade of the protein tissue inhibitor of metalloproteinase 1 can ameliorate myocarditis and its consequences; this observation may be of substantial clinical usefulness. Finally, our studies of infection of the CNS by coxsackievirus B3 in neonates indicated that the virus may preferentially infect stem cells and be carried into the brain parenchyma by these cells as the cells migrate toward their final destinations.

AUTOIMMUNITY

Together with colleagues at the University of Utah and at La Jolla Institute of Allergy and Immunology, we are studying the molecular basis of autoimmunity induced by viral infection. Some autoimmune diseases (e.g., multiple sclerosis) appear to be triggered and/or exacerbated by a wide variety of viral infections. Two general mechanisms, molecular mimicry and bystander activation, have been proposed to explain this phenomenon. We have suggested an alternative explanation that is based on changes in antigen presentation that occur during almost all viral infections.

PUBLICATIONS

Botten, J., Alexander, J., Pasquetto, V., Sidney, J., Barrowman, P., Ting, J., Peters, B., Southwood, S., Stewart, B., Rodriguez-Carreno, M.P., Mothe, B., Whitton, J.L., Sette, A., Buchmeier, M.J. Identification of protective Lassa virus epitopes that are restricted by HLA-A2. *J. Virol.* 80:8351, 2006.

Botten, J., Whitton, J.L., Barrowman, P., Sidney, J., Whitmire, J.K., Alexander, J., Ting, J., Bui, H.-H., Sette, A., Buchmeier, M.J. HLA-A2-restricted protection against lethal lymphocytic choriomeningitis. *J. Virol.* 81:2307, 2007.

Cornell, C.T., Kiosses, W.B., Harkins, S., Whitton, J.L. Inhibition of protein trafficking by coxsackievirus B3: multiple viral proteins target a single organelle. *J. Virol.* 80:6637, 2006.

Crocker, S.J., Whitmire, J.K., Frausto, R.F., Chertboonmuang, P., Soloway, P.D., Whitton, J.L., Campbell, I.L. Persistent macrophage/microglial activation and myelin disruption after experimental autoimmune encephalomyelitis in tissue inhibitor of metalloproteinase-1-deficient mice. *Am. J. Pathol.* 169:2104, 2006.

Fernandez-Borges, N., Brun, A., Whitton, J.L., Parra, B., Diaz-San Segundo, F., Salguero, F.J., Torres, J.M., Rodriguez, F. DNA immunization can break immunological tolerance to PrP in wild-type mice and attenuates prion disease after intracerebral challenge. *J. Virol.* 80:9970, 2006.

Feuer, R., Whitton, J.L. Preferential coxsackievirus replication in proliferating/activated cells: implications for virus tropism, persistence, and pathogenesis. *Curr. Top. Microbiol. Immunol.*, *in press*.

Hunziker, I.P., Cornell, C.T., Whitton, J.L. Deletions within the 5' UTR of coxsackievirus B3: consequences for virus translation and replication. *Virology* 360:120, 2007.

Libbey, J.E., Tsunoda, I., Whitton, J.L., Fujinami, R.S. Infectious RNA isolated from the spinal cords of mice chronically infected with Theiler's murine encephalomyelitis virus. *J. Virol.* 81:3009, 2007.

Whitton, J.L. Infection: immunological barriers. *In: Encyclopedia of Life Sciences* [online]. Wiley, New York. Published April 16, 2007. doi:10.1002/9780470015902.a0000940.pub2.

Laboratory of Translational Neurophysiology and the San Diego Substance Abuse and Minorities Project

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The current and main focus of the laboratory is to determine the CNS etiology of substance abuse. Our strategy in studying these disorders in patients has been to capitalize on the use of new accurate structured diagnostics, physiologic measures, and the latest genetic techniques to identify risk and protective factors for the disorders of alcohol and drug dependence. Because the prevalence of substance dependence varies among certain racial/ethnic groups, we have also focused on studying a wide range of ethnic groups to evaluate genetic and cultural differences that may lead to new clues for the causes of the disorders. This work encompasses parallel studies in animal models, currently called "translational research." This type of research allows investigators to simultaneously evaluate disorders in patients and model the condition in animals so that progress toward understanding the causes of these disabilities can be more rapidly pursued.

The differences in prevalence rates of alcohol and drug use and abuse between ethnic groups, as well as between different strains of rats, provides an opportunity to investigate how genetic variation may influence substance use and abuse. One difference between ethnic groups is a natural variation in the genes that encode the structure of the enzymes that metabolize alcohol. We were the first to identify a role for genetic variations in 2 genes, the gene for alcohol dehydrogenase

(*ADH1B*3*) and the gene for cytosolic aldehyde dehydrogenase (*ALDH1A*1*), in African Americans, Southwest California Indians, and islanders on Trinidad and Tobago. We have also shown that individuals of East Indian descent with the gene for another variant in alcohol dehydrogenase (*ADH1C2*2*) are more at risk for alcoholism and alcoholic liver disease than are individuals without that gene.

Although variations in the metabolism of alcohol clearly affect the risk for alcohol dependence, other genes also influence the development of this disorder. To identify these genes, we did a genome scan in Southwest California Indian families for alcoholism and behaviors related to substance abuse. We found that several sites in the genome were linked not only to multiple drugs of abuse but also to body mass, suggesting that the same selective pressure may have enriched for genetic variants that increase the risk for consumption of both food and drugs of abuse.

One crucial variable in the development of alcohol dependence is the time at which an individual begins to consume alcohol. We found that alcohol dependence was 5–6 times more likely in youth who starting drinking before age 13 years than in individuals who did not start drinking until after age 16 years. Studying biobehavioral risk factors for and the consequences of underage alcohol use in young adults and in animal models is critical for several reasons. First, rapid development of alcohol dependence is more likely in underage drinkers than in older drinkers, suggesting that alcohol may be “more addicting” to underage drinkers, a finding also partly confirmed by our controlled studies in animal models of the disorder.

Second, exposure to alcohol and other substances during adolescence may have long-lasting consequences. Rapid changes in neural organization occur during this period, and these changes in CNS organization may make the brain uniquely vulnerable to injury by drug use or abuse. Thus, during adolescence, drugs may be both more addicting and more neurotoxic, a combination that makes drug abuse particularly malignant for adolescents. To combat this problem, we not only are conducting more research into the etiology of underage drinking but also are developing new strategies, called “environmental preventions,” to reduce underage drinking in high-risk populations.

PUBLICATIONS

Ehlers, C.L. Variations in ADH and ALDH in Southwest California Indians. *Alcohol Res. Health* 30:14, 2007.

Ehlers, C.L., Hurst, S., Phillips, E., Gilder, D.A., Dixon, M., Gross, A., Lau, P., Yehuda, R. Electrophysiological responses to affective stimuli in American Indians experiencing trauma with and without PTSD. *Ann. N. Y. Acad. Sci.* 1071:125, 2006.

Ehlers, C.L., Montane-Jaime, K., Moore, S., Shafe, S., Joseph, R., Carr, L. Association of the *ADH1B*3* allele with alcohol-related phenotypes in Trinidad. *Alcohol Clin. Exp. Res.* 31:216, 2007.

Ehlers, C.L., Phillips, E. Association of EEG alpha variants and alpha power with alcohol dependence in Mexican American young adults. *Alcohol* 41:13, 2007.

Ehlers, C.L., Phillips, E., Finnerman, G., Gilder, D.A., Lau, P., Criado, J. P3 components and adolescent binge drinking in Southwest California Indians. *Neurotoxicol. Teratol.* 29:153, 2007.

Ehlers, C.L., Slutske, W., Gilder, D.A., Lau, P. Age of first marijuana use and the occurrence of marijuana use disorders in Southwest California Indians. *Pharmacol. Biochem. Behav.* 86:290, 2007.

Ehlers, C.L., Slutske, W., Gilder, D.A., Lau, P., Wilhelmson, K.C. Age at first intoxication and the development of alcohol use disorders in Southwest California Indians. *Alcohol Clin. Exp. Res.* 30:1856, 2006.

Ehlers, C.L., Walker, B.M., Pian, J.P., Roth, J.L., Slawecki, C.J. Increased alcohol drinking in isolate-housed alcohol-preferring rats. *Behav. Neurosci.* 121:111, 2007.

Ehlers, C.L., Wall, T.L., Corey, L., Lau, P., Gilder, D.A., Wilhelmson, K.C. Heritability of illicit drug use and transition to dependence in Southwest California Indians. *Psychiatr. Genet.* 17:171, 2007.

Ehlers, C.L., Wilhelmson, K.C. Genomic screen for substance dependence and body mass index in Southwest California Indians. *Genes Brain Behav.* 6:184, 2007.

Gilder, D.A., Lau, P., Corey, L., Ehlers, C.L. Co-morbidity of alcohol dependence with other psychiatric disorders in young adult Mexican Americans. *J. Addict. Dis., in press.*

Gilder, D.A., Lau, P., Corey, L., Ehlers, C.L. Factors associated with remission from cannabis dependence in Southwest California Indians. *J. Addict. Dis., in press.*

Gilder, D.A., Lau, P., Dixon, M., Corey, L., Phillips, E., Ehlers, C.L. Co-morbidity of select anxiety, affective, and psychotic disorders with cannabis dependence in Southwest California Indians. *J. Addict. Dis.* 25:67, 2006.

Montane-Jaime, K., Moore, S., Shafe, S., Joseph, R., Crooks, H., Carr, L., Ehlers, C.L. *ADH1C*2* allele is associated with alcohol dependence and elevated liver enzymes in Trinidad and Tobago. *Alcohol* 39:81, 2006.

Moore, S., Montane-Jaime, K., Carr, L.G., Ehlers, C.L. Variations in alcohol-metabolizing enzymes in people of East Indian and African descent from Trinidad and Tobago. *Alcohol Res. Health* 30:28, 2007.

Moore, S., Montane-Jaime, K., Shafe, S., Joseph, R., Crooks, H., Carr, L., Ehlers, C.L. Association of *ALDH1* promoter polymorphisms with alcohol-related phenotypes in Trinidad and Tobago. *J. Stud. Alcohol Drugs* 68:192, 2007.

Slawecki, C.J. Two-choice reaction time performance in Sprague-Dawley rats exposed to alcohol during adolescence or adulthood. *Behav. Pharmacol.* 17:605, 2006.

Slawecki, C.J., Roth, J., Gilder, A. Neurobehavioral profiles during the acute phase of ethanol withdrawal in adolescent and adult Sprague-Dawley rats. *Behav. Brain Res.* 170:41, 2006.

Thorsell, A., Ehlers, C.L. Neuropeptide Y in brain function. *In: Neuroactive Proteins and Peptides*, 3rd ed. Lim, R. (Ed.). Springer, New York, 2006, p. 523. *Handbook of Neurochemistry and Molecular Neurobiology*. Lajtha, A. (Ed.).

Thorsell, A., Slawecki, C.J., El Khoury, A., Mathe, A.A., Ehlers, C.L. The effects of social isolation on neuropeptide Y levels, exploratory and anxiety-related behaviors in rats. *Pharmacol. Biochem. Behav.* 83:28, 2006.

Venner, K.L., Wall, T.L., Lau, P., Ehlers, C.L. Testing of an orthogonal measure of cultural identification with adult Mission Indians. *Cultur. Divers. Ethnic Minor. Psychol.* 12:632, 2006.

Cellular and Molecular Mechanisms of Neuronal Signaling in the CNS

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DEVELOPMENTAL REGULATION OF ION CHANNEL FUNCTION IN CNS NEURONS

CNS neurons express a variety of ion channels and membrane receptors that generate the complex patterns of electrical activity that underlie all brain functions. Intracellular second messenger pathways play an essential role in this process by controlling the activity of specific classes of ion channels. Calcium ions are one of the most important intracellular second messengers in CNS neurons; calcium is abundant outside the neuron but highly regulated in the intracellular compartment. The levels of intracellular calcium can be altered by several mechanisms, such as the activity of voltage-gated calcium ion channels, which provide a pathway for calcium to enter the neuron, or the activation of transmitter receptors such as type 1 metabotropic glutamate receptors (mGluR1s) that control release of calcium from intracellular stores.

The mGluR1s are expressed at excitatory synapses in the CNS and are particularly abundant in the dendrites of adult cerebellar Purkinje neurons, where their role in synaptic physiology and neuronal excitability has been extensively studied. These receptors are also expressed in developing Purkinje neurons and play a prominent role in the regulating the development of these neurons and in contributing to synaptic physiology and neuronal excitability. However, little is known about the physiologic responses produced by mGluR1 activation in immature Purkinje neurons early in development. We used simultaneous recordings of membrane potential and intracellular levels of calcium in immature cultured Purkinje neurons at different stages of development to address this question.

We found that mGluR1 activation produced a prominent increase in intracellular calcium at all stages of development of Purkinje neurons; the largest increase occurred early in development. Interestingly, a fast

membrane hyperpolarization was associated with the increased calcium levels in the immature neurons but was not evident in similar experiments in mature cultured Purkinje neurons. The fast hyperpolarization varied in amplitude with membrane potential and intracellular calcium levels and was blocked by apamin, an antagonist of small conductance, calcium ion-activated potassium ion channels (SK channels), indicating that these channels are the mediator of the fast membrane hyperpolarization. Thus, mGluR1 activation and the resulting release of calcium from intracellular stores and activation of SK channels represents a mechanism through which mGluR1 can modulate neuronal excitability and the patterns of electrical activity of Purkinje neurons early in development before the expression of later-developing mechanisms such as inhibitory synaptic transmission.

CNS NEUROINFLAMMATION

Recent studies have shown that CNS neurons and astrocytes produce chemical factors, such as cytokines and chemokines, previously thought to function only in the immune system, and to express receptors for these factors. These results implicate a role for cytokines and chemokines as signaling molecules in the CNS. Studies have indicated that a hallmark of several CNS inflammatory and neurodegenerative diseases is elevated CNS levels of the chemokine CXCL10, suggesting a key role for CXCL10 in these conditions. Neurons express receptors for CXCL10 and may be an important target of this chemokine. Little is known about the actions of CXCL10 on CNS neurons. To address this question we determined if signal transduction pathways known to play a central role in CNS neuronal physiology are affected by exposure to CXCL10.

Results of Western blot analyses indicated that prolonged exposure to CXCL10 to simulate chronic neuroinflammatory conditions activated extracellular signal-regulated kinase 1/2 in hippocampal neuronal cultures. In addition, the downstream effectors of the kinase, transcriptional factors CREB and NF- κ B, were also altered by prolonged exposure to CXCL10. CREB and NF- κ B play critical roles in CNS processes such as learning and memory, neuronal plasticity, neurodegeneration, and neuronal development. Therefore, these functions could be important targets of CXCL10 actions during neuroinflammation.

PUBLICATIONS

Sabeti, J., Nelson, T.E., Purdy, R.H., Gruol, D.L. Steroid pregnenolone sulfate enhances NMDA-receptor-independent long-term potentiation at hippocampal CA1 synapses: role for L-type calcium channels and sigma-receptors. *Hippocampus* 17:349, 2007.

Vereyken, E.J.F., Bajova, H., Chow, S., de Graan P.N.E., Gruol, D.L. Chronic interleukin-6 alters the level of synaptic proteins in hippocampus in culture and in vivo. *Eur. J. Neurosci.* 25:3605, 2007.

Role of the Neuregulins in the Nervous System

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The focus of our research is understanding the signaling mechanisms that underlie the establishment and maintenance of mature neuronal and glial cell phenotypes. We are studying the roles played by a subfamily of receptor protein-tyrosine kinases, the ErbBs (EGFR, ErbB2, ErbB3, and ErbB4), and their ligands, the neuregulins (NRG-1–NRG-4). NRG-1 was first recognized as the Schwann cell mitogen glial growth factor. NRG-1 was also termed ARIA (for acetylcholine receptor inducing activity), which was thought to regulate expression of acetylcholine receptors at developing neuromuscular junctions. These distinct functions are now thought to be served by discrete types of NRG-1 (I, II, and III) that arise by alternative splicing. A primary goal of our research is to understand the specific roles of each of these types of NRG-1 in the nervous system.

NRG-1 supports survival of Schwann cells and regulates the number of premyelinating Schwann cells. The results of genetic studies suggested that the type III isoform serves in this capacity, and we have helped determine that this isoform also plays a key role in regulating the thickness of the myelin sheath. The emerging picture is that different NRG-1 isoforms serve as signaling molecules from neuron to glial cell and from neuron to muscle to carry out distinct biological activities. We are also pursuing the roles of these NRG-1 isoforms in the brain, which became an area of considerable interest after NRG-1 was identified as a susceptibility gene for schizophrenia.

We have 4 areas of primary interest. The first is the roles of the 3 types of NRG-1 in the developing and mature nervous system. We developed transgenic mice that permit the tetracycline-regulated expression of specific NRG-1 isoforms. With these mice, we can assess the distinct biological functions served by each isoform.

The second area is neurogenesis and migration. We found that the neuregulin receptor ErbB4 is expressed by multiple tangentially migrating populations of neuronal cells in the developing and mature nervous sys-

tem. ErbB4 is expressed at high levels in the mature subventricular zone and rostral migratory stream, one of the few regions in the brain in rats where neurogenesis occurs in adults. We are searching for the endogenous ligands and testing the effects of the NRGs on cells derived from the subventricular zone. Our data suggest that ErbB4 influences both the proliferation of neural progenitor cells and migration of neuroblasts in the rostral migratory stream.

The third area of interest is the effects of the loss of ErbB4 function in the mature brain. We are analyzing the phenotype of mice that lack the gene for ErbB4 in the nervous system. These animals have a reduction in anxiety-like behavior, and we are testing the hypothesis that the loss of ErbB4 in the amygdala underlies this defect. Our current findings suggest that the chemical inhibition of ErbB4 in this brain region mimics the genetic loss of function.

Last, we are developing novel, bacterial artificial chromosome-based transgenic tools that permit regulated gene expression in specific subsets of neurons. We have successfully developed lines of mice that permit regulated gene expression in cholinergic neurons, and we are evaluating similar lines that permit regulated expression in the medium spiny neurons of the striatum. These animal models may be useful for studies of neural development and neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases.

PUBLICATIONS

Funes, M., Miller, J.K., Lai, C., Carraway, K.L. III, Sweeney, C. The mucin Muc4 potentiates neuregulin signaling by increasing the cell-surface populations of ErbB2 and ErbB3. *J. Biol. Chem.* 281:19310, 2006.

Ghashghaei, H.T., Lai, C., Anton, E.S. Neuronal migration in the adult brain: are we there yet? *Nat. Rev. Neurosci.* 8:141, 2007.

Ponomareva, O.N., Fischer, T.M., Lai, C., Rimer, M. Schwann cell-derived neuregulin-2 α can function as a cell-attached activator of muscle acetylcholine receptor expression. *Glia* 54:630, 2006.

Role of Galanin Receptors in Antidepressant and Anxiolytic Actions

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Galanin, a neuropeptide first isolated from intestine, regulates many functions of the CNS through 3 G protein-coupled receptors:

GalR1–GalR3. The extensive overlapping between the galaninergic system and monoamine pathways fostered our interest in studying the role of galanin in mood regulation. We found that galanin and GalR2 are upregulated after treatment with antidepressant agents such as fluoxetine and desipramine or treatment with electroconvulsive shock, suggesting a possible involvement of the galanin system in antidepressant actions. Administration of the synthetic galanin receptor agonists galanin and galmic had an antidepressant-like effect in rodent models.

We are defining GalR2 as a putative drug target for a new class of antidepressant drugs. Activation of GalR2 by the GalR2-selective agonist galanin (2-11) enhances serotonergic signaling and leads to an increase in serotonin release in both the dorsal raphe nucleus and the ventral part of the hippocampus in rats. In addition, GalR2 signaling promotes hippocampal neurogenesis in adult mice, an effect associated with most clinically effective antidepressant treatments in humans, by effects on the proliferation and survival of newly generated neurons.

GalR3 is another important galanin receptor involved in mood regulation. We have shown that a high-affinity selective GalR3 antagonist, 3-(3,4-dichlorophenylimino)-1-(6-methoxy-pyridin-3-yl)indolin-2-one, has an antidepressant-like effect in mice and rats, similar to the effect of desipramine. Despite the observed behavioral effect, little is known about the distribution and physiology of GalR3. Thus, a major objective is to understand the underlying molecular mechanisms that mediate the antidepressant and anxiolytic effect of the GalR3 antagonist. For this purpose, we are using mice deficient in both GalR1 and GalR2 to label the neuronal populations activated by galanin, presumably through GalR3 signaling, and to study the effects of site-specific injection of galanin on depression-related behaviors.

In ongoing studies, we are developing and characterizing synthetic high-affinity GalR2 agonists and GalR3 antagonists, designed and synthesized through collaborative work with E. Roberts, Department of Chemistry, and J. Rebeck, Skaggs Institute for Chemical Biology.

PUBLICATIONS

Barr, A.M., Kinney, J.W., Hill, M.N., Lu, X., Biros, S., Rebeck, J., Jr., Bartfai, T. A novel, systemically active, selective galanin receptor-3 ligand exhibits antidepressant-like activity in preclinical tests. *Neurosci. Lett.* 405:111, 2006.

Lu, X., Sharkey, L., Bartfai, T. The brain galanin receptors: targets for novel antidepressant drugs. *CNS Neurol. Disord. Drug Targets* 6:183, 2007.

Shi, T.J., Hua, X.Y., Lu, X., Malkmus, S., Kinney, J., Holmberg, K., Wirz, S., Cecatelli, S., Yaksh, T., Bartfai, T., Hokfelt, T. Sensory neuronal phenotype in galanin receptor 2 knockout mice: focus on dorsal root ganglion neuron development and pain behaviour. *Eur. J. Neurosci.* 23:627, 2006.

Restless Legs Syndrome and Event-Related Brain Potentials

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Restless legs syndrome may be a condition of impaired CNS dopamine function. Dopamine deficiency is associated with bradykinesia and impaired attention (e.g., Parkinson's disease and attention deficit disorder), and dopamine restoration or excess (e.g., treatment of patients with Parkinson's disease with L-3,4-dihydroxyphenylalanine [L-dopa] and of patients with attention deficit disorder with amphetamine) is associated with normal or excessive movement and with improved attention. The signs and symptoms of restless legs syndrome are decreased by movement, especially by walking. Some patients also report that increased mental activity, focused concentration, or being distracted decrease the manifestations of the syndrome.

Event-related brain potentials (ERPs) were used to assess patients with restless legs syndrome. ERPs enable the direct evaluation of CNS neuroelectric activity during processing of stimulus information. The P3a and P3b subcomponents of the P300 ERP reflect the operation of frontal attentional and temporoparietal memory operations that appear to be associated with dopaminergic and variation in locus coeruleus norepinephrine pathways, respectively. A 3-stimulus oddball task or choice task can be used to elicit both P3a and P3b by presenting stimuli once every 2 seconds in a series. The infrequently occurring target stimuli are to be detected and responded to in the context of more frequently occurring standard stimuli, and the stimuli are difficult to discriminate (e.g., circles 4.0 and 3.5 cm in diameter). When an infrequently presented "distracter" stimulus randomly occurs (e.g., a large checkerboard pattern), the attentional focus induced by the discrimination task is disrupted and produces a P3a potential that has its maximum amplitude over frontal-central areas of the scalp; the target stimulus elicits the P3b potential over the parietal areas. This difference in scalp topography is hypothesized to reflect distinct neural generating systems related to attention and memory processing of the stimuli.

Figure 1 illustrates scalp topography amplitudes from the application of a visual 3-stimulus task to unaffected controls, patients with restless legs syndrome, and a group of patients with Parkinson's disease. Of note, P3a amplitude from the distracter

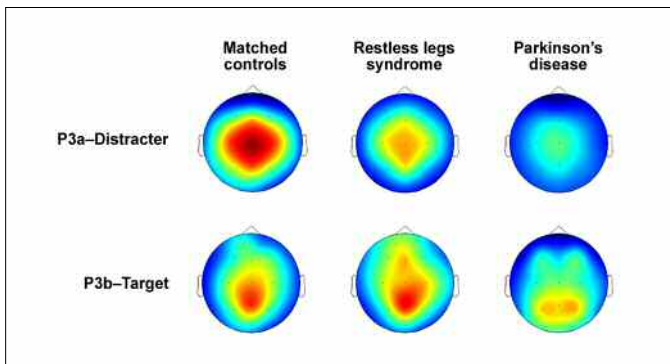


Fig. 1. Topographic scalp amplitude mappings from the distracter and target stimuli. Each participant in the 3 groups (7 per group), controls, patients with restless legs syndrome, and patients with Parkinson's disease, performed the same task. The patients were matched for age, sex, and education. The P3a amplitudes illustrate increasing dopaminergic deficits from left to right; the P3b amplitudes differed little between the control group and the patients with restless legs syndrome; patients with Parkinson's disease had appreciably smaller amplitudes overall.

stimulus decreases as dopaminergic activity changes from normal to an increased activity associated with neurologic disease. P3b amplitude from the target stimulus is relatively similar between the control group and the patients with restless legs syndrome but decreases appreciably in the more neurologically damaged patients with Parkinson's disease. These results suggest that ERPs can be used to track dopaminergic changes in patients. If substantiated with increased numbers of subjects, the P3a may be useful as a neuroelectric marker of the efficacy of drug treatments.

PUBLICATIONS

Azizian, A., Polich, J. Evidence for attentional gradient in the serial position memory curve from ERPs. *J. Cogn. Neurosci.*, *in press*.

Cano, M.E., Class, Q.A., Polich, J. Affective valence, stimulus attributes, and P300: color vs black/white and normal vs scrambled images. *Int. J. Psychophysiol.*, *in press*.

Conroy, M.A., Polich, J. Affective valence and P300 when stimulus arousal level is controlled. *Cogn. Emot.* 21:891, 2007.

Conroy, M.A., Polich, J. Normative variation of P3a and P3b from a large sample: gender, topography, and response time. *J. Psychophysiol.* 21:22, 2007.

Gonsalvez, C.J., Barry, R.J., Rushby, J.A., Polich, J. Target-to-target interval, intensity, and P300 from an auditory single-stimulus task. *Psychophysiology* 44:245, 2007.

Olofsson, J.K., Polich, J. Affective visual event-related potentials: arousal, repetition, and time-on-task effects. *Biol. Psychol.* 75:101, 2007.

Polich, J. Updating P300: an integrative theory of P3a and P3b. *Clin. Neurophysiol.* 118:2128, 2007.

Rossini, P.M., Rossi, S., Babiloni, C., Polich, J. Clinical neurophysiology of aging brain: from normal aging to neurodegeneration. *Prog. Neurobiol.*, *in press*.

Mouse Behavioral Assessment Core Facility and Alcohol and Drug Self-administration

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MOUSE BEHAVIORAL ASSESSMENT CORE FACILITY

We continue to run the Mouse Behavioral Assessment Core facility and are part of the La Jolla Interdisciplinary Neuroscience Center Cores, funded by the National Institutes of Health Blueprint Initiative. The purpose of the facility is to provide high-quality mouse behavioral assessments to neuroscientists located near Scripps Research. A primary focus is to provide tests that allow investigators to make transitions from laboratory findings to clinical applications by enabling the modeling of human diseases and the development of treatment strategies. For example, test batteries have been developed for several neuropsychiatric disorders, including anxiety disorders, depressive disorders, disorders of learning and memory, disorders of motor functioning, drug and alcohol abuse and dependence, eating disorders, and other compulsive and impulsive disorders.

This year we added a comprehensive laboratory animal monitoring system that allows continuous assessment of activity, feeding, drinking, body temperature, and measures of metabolism. Recently, we have provided services for investigators in the Departments of Molecular and Experimental Medicine, Cell Biology, and Chemistry and in the Molecular and Integrative Neurosciences Department. In addition, several investigators from outside institutions have used our services.

ALCOHOL AND DRUG SELF-ADMINISTRATION

We are using mouse models to investigate the neural bases of behavior, particularly motivated behaviors such as drug and alcohol self-administration and exploratory drive. As part of a multisite integrated neuroscience initiative on alcoholism sponsored by the National Institute on Alcohol Abuse and Alcoholism, we have developed models of excessive alcohol drinking after a period of abstinence in alcohol-dependent mice. We have used these models in both genetic and neuropharmacologic experiments. Currently, in collaboration with other investigators at Scripps Research, we are testing several mutant mouse strains. The focus of the initiative is excessive alcohol drinking and the role of the extended

amygdala; therefore, we have been perfecting our implantation of intracerebral cannulas in mice for site-specific administration of test compounds.

Another focus of our group is self-administration of intravenous cocaine, morphine, and methamphetamine in mice. We are studying the initial phases of drug addiction, and recently we developed a high-throughput screening protocol for use in genetic and pharmacologic experiments. We have also developed a model of relapse to drug-seeking behavior in mice. Understanding the underlying neural mechanisms of the initiation of and relapse to drug seeking can enhance the ability to treat and prevent addictive disorders.

PUBLICATIONS

Chu, K., Koob, G.F., Cole, M., Zorrilla, E.P., Roberts, A.J. Dependence-induced increases in ethanol self-administration in mice are blocked by the CRF₁ receptor antagonist antalarmin and by CRF₁ receptor knockout. *Pharmacol. Biochem. Behav.* 86:813, 2007.

Finn, D.A., Snelling, C., Fretwell, A.M., Tanchuck, M.A., Underwood, L., Cole, M., Crabbe, J.C., Roberts, A.J. Increased drinking during withdrawal from intermittent ethanol exposure is blocked by the CRF receptor antagonist D-Phe-CRF(12-41). *Alcohol. Clin. Exp. Res.* 31:939, 2007.

Kang-Park, M.H., Kieffer, B.L., Roberts, A.J., Siggins, G.R., Moore, S.D. Presynaptic δ opioid receptors regulate ethanol actions in central amygdala. *J. Pharmacol. Exp. Ther.* 320:917, 2007.

Kunz, S., Rojek, J.M., Roberts, A.J., McGavern, D.B., Oldstone, M.B.A., de la Torre, J.-C. Altered central nervous system gene expression caused by congenitally acquired persistent infection with lymphocytic choriomeningitis virus. *J. Virol.* 80:9082, 2006.

Metten P, Buck, K.J., Merrill, C.M., Roberts, A.J., Yu, C.-H., Crabbe, J.C. Use of a novel mouse genotype to model acute benzodiazepine withdrawal. *Behav. Genet.* 37:160, 2007.

Newton, P.M., Kim, J.A., McGeehan, A.J., Paredes, J.P., Chu, K., Wallace, M.J., Roberts, A.J., Hodge, C.W., Messing, R.O. Increased response to morphine in mice lacking protein kinase C epsilon. *Genes Brain Behav.* 6:329, 2007.

Prut, L., Abramowski, D., Krucker, T., Levy, C.L., Roberts, A.J., Staufenbiel, M., Wiessner, C. Aged APP23 mice show a delay in switching to the use of a strategy in the Barnes maze. *Behav. Brain Res.* 179:107, 2007.

Microarray and Electrophysiologic Investigations of Adaptive and Maladaptive Neuronal Plasticity

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As part of our effort to apply microarray-based strategies to studies of the neurobiology of drug abuse, we profiled gene expression in reward-related regions of the brain in rats after chronic intermittent administration of alcohol to induce dependence.

Intermittent exposure to alcohol in animal models mimics binge patterns of alcohol abuse in humans and induces dependence more rapidly than does continuous administration. Gene expression was profiled in 3 brain regions involved in the reinforcing actions of alcohol: the medial prefrontal cortex, the nucleus accumbens, and the amygdala. For these studies, we used high-density oligonucleotide microarrays with a virtual genome-wide coverage.

We found that a member of the endogenous family of protein kinase A inhibitors, PKI- α , was increased in all brain regions tested. Concomitantly, we observed a downregulation of several protein kinase A-regulated transcripts in the brain regions studied. These results support the notion that adaptations of the protein kinase A pathway play a role in the central effects of alcohol dependence.

In studies on the role of the hypothalamus in the neurobiology of drug abuse, we profiled gene expression in 4 hypothalamic regions to characterize the regional gene expression repertoire of the hypothalamus. The hypothalamus plays a central role in the regulation of feeding, stress, reward, and visceral functions. Previously, using microarray analysis, we found a profound reprogramming of gene expression in the lateral hypothalamus of rats with escalated patterns of cocaine self-administration. More recently, we used laser microdissection, 2 rounds of in vitro transcription, and high-density microarrays that allow whole-genome coverage to study gene expression in nuclei of the hypothalamus. We examined the suprachiasmatic nucleus, the paraventricular nucleus, the anterior hypothalamic nucleus, and the lateral hypothalamic area in samples from individual animals of both sexes. The results indicated that gene expression in the suprachiasmatic nucleus is most similar to expression in the paraventricular nucleus and that gene expression in the anterior hypothalamic nucleus is most similar to expression in the lateral hypothalamic area. The rat homologs of the homeobox genes 3 and 6 for the *Drosophila* transcription factor sine oculis were highly selectively expressed in the suprachiasmatic nucleus and may be used as molecular markers for this nucleus.

We also characterized a novel form of long-term potentiation (LTP) in the juxtacapsular subdivision of the bed nucleus of the stria terminalis (jcBNST) that we recently discovered and that is impaired in rats with a history of dependent drug intake. The BNST has been implicated in stress responses and in the motivational dysregulation associated with drug dependence. We

observed that this LTP is characterized by a long-lasting change in the intrinsic excitability of jcBNST neurons because of a shift to hyperpolarization of the threshold for the generation of action potentials.

The activity-dependent decrease in the firing threshold of jcBNST neurons was mediated by changes in D-type potassium current. DNA microarray analysis indicated that the expression of Kv1.2, a member of the family of potassium channels implicated in mediating the D-type current, was significantly increased in the jcBNST with impaired LTP in rats with a history of alcohol, cocaine, or heroin dependence. These findings suggest that increased density of Kv1.2 subsequent to its increased gene expression may contribute to the refractoriness to LTP in animals with histories of drug dependence.

PUBLICATIONS

Conti, B., Maier, R., Barr, A.M., Morale, M.C., Lu, X., Sanna, P.P., Bilbe, G., Hoyer, D., Bartfai, T. Region-specific transcriptional changes following the three antidepressant treatments: electro convulsive therapy, sleep deprivation and fluoxetine. *Mol. Psychiatry* 12:167, 2007.

Repunte-Canonigo, V., Lutjens, R., van der Stap, L.D., Sanna, P.P. Increased expression of protein kinase A inhibitor α (PKI- α) and decreased PKA-regulated genes in chronic intermittent alcohol exposure. *Brain Res.* 1138:48, 2007.

Thorsell, A., Repunte-Canonigo, V., O'Dell, L.E., Chen, S.A., King, A.R., Lekic, D., Koob, G.F., Sanna, P.P. Viral vector-induced amygdala NPY overexpression reverses increased alcohol intake caused by repeated deprivations in Wistar rats. *Brain* 130:1330, 2007.

Cellular Physiology of Brain Cannabinoids and Peptides

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NEUROBIOLOGY OF CANNABINOID SUBSTANCES

Cannabinoid substances contained in marijuana have powerful psychoactive properties and alter cognitive processes via activation of cannabinoid-1 (CB₁) receptors, but in order to function properly, the brain produces its own cannabinoid ligands. Our objectives are to uncover the cellular mechanisms that underlie the central effects of cannabinoid ligands and to determine the role played by endogenous cannabinoids.

Using a physiologic approach, we are investigating the modulation of synaptic transmission and plasticity. In this approach, we record from neurons in brain tissue from the hippocampus and neocortex, 2 structures involved in learning and memory, and from the amygdala, a part of the brain implicated in addictive behaviors. In collaboration with D. Piomelli, University of California, Irvine, we are using various pharmacologic tools to study the routes of degradation of endogenous cannabinoids. In our collaborations with B. Lamboloz and J. Rossier of France, we are using single-cell reverse transcriptase-polymerase chain reaction after whole-cell recording to characterize the neuronal populations that express transcripts for CB₁ receptors and other effectors of the endogenous cannabinoid system.

We found that endogenous cannabinoids acting at CB₁ receptors in the hippocampus selectively decrease excitatory transmission and restrict synaptic plasticity. We also discovered that cyclooxygenase-2 has a predominant role in controlling the tonic level of endogenous cannabinoids that modulate synaptic activity and plasticity. Consistent with such a role for endogenous cannabinoids, our research in collaboration with Dr. Lamboloz indicated that more than half of pyramidal neurons express CB₁ receptors in the neocortex. The results of physiologic experiments confirmed that CB₁ receptors modulate neocortical networks.

Our findings bring new light to the physiologic role of endogenous cannabinoids in synaptic transmission and clarify their role in synaptic plasticity. Our results also provide further evidence of the tonic role of CB₁ receptor ligands in the regulation of neuronal activity in the forebrain and reinforce the idea of the primary role of these ligands at glutamatergic synapses.

NEUROPEPTIDES AND ALCOHOL

Neuropeptides are found throughout the brain and strongly influence neuronal activity. Peptides such as corticotropin-releasing factor (CRF) and nociceptin are involved in the CNS effects of ethanol. In collaborations with M. Roberto, Committee on the Neurobiology of Addictive Disorders, and G.R. Siggins, Molecular and Integrative Neurosciences Department, we are examining the actions of ethanol in the central amygdala, a brain region prominently involved in alcohol dependence and reinforcement. Our results indicate that CRF₁ receptors mediate the ethanol enhancement of inhibitory transmission, providing a cellular mechanism for the involvement of CRF in the effects of ethanol and supporting a role for the peptide in the motivational effects of ethanol.

Ethanol also decreases glutamatergic transmission in the central amygdala, suggesting that chronic ethanol treatment and withdrawal lead to neuroadaptations of glutamatergic transmission at both presynaptic and postsynaptic sites in the central amygdala. The complex modulation of synaptic transmission by ethanol may contribute to ethanol intoxication, reinforcement, tolerance, and dependence. CRF₁ receptors could be an important therapeutic target for the treatment of stress-induced alcohol drinking.

PUBLICATIONS

Hill, E.L., Gallopin, T., F  r  zou, I., Cauli, B., Rossier, J., Schweitzer, P., Lambolez, B. Functional CB₁ receptors are broadly expressed in neocortical GABAergic and glutamatergic neurons. *J. Neurophysiol.* 97:2580, 2007.

Neurotransmission, Neuropeptides, and Drugs of Abuse

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We investigate the role of neuropeptides and the actions of abused drugs on electrophysiologic and molecular mechanisms of neuronal and synaptic function. We use extracellular, intracellular, and patch recording of brain neurons *in vitro*. We treat neurons with transmitters, peptides, drugs, and neurotoxins by micropipettes and by superfusion, and we activate synaptic transmission via stimulating electrodes. We also use molecular methods to assess drug-induced alterations of receptors.

We investigate synaptic mechanisms and peptide and drug effects in 2 brain regions, the nucleus accumbens and the central amygdala, because these regions are involved in stress and drug abuse. In previous studies, we discovered inhibitory effects of opioid peptides on synaptic transmission in the hippocampus and nucleus accumbens. Patch-clamp studies of neurons in the central amygdala indicated that the opioid-like peptide nociceptin decreases presynaptic vesicular release of the inhibitory transmitter γ -aminobutyric acid (GABA) and reverses the effect of ethanol in enhancing the release of GABA. Both δ and μ opioid receptor agonists also

reduce GABA release in neurons in the central amygdala, with little postsynaptic effect.

Our previous findings also suggested that glutamatergic synapses, especially receptors for *N*-methyl-D-aspartate (NMDA), play a role in opiate and alcohol dependence. Chronic morphine treatment altered several pharmacologic and molecular properties of NMDA receptor-mediated excitatory postsynaptic potentials (EPSPs) in the nucleus accumbens and central amygdala, suggesting changes in the function and/or composition of the subunits of NMDA receptors. Our studies with quantitative polymerase chain reaction and Western blots of NMDA receptor subunits indicate that chronic morphine does not change mRNA for the 3 major subunits, NR1, NR2A, and NR2B, in the nucleus accumbens, but protein levels for NR1 and NR2B increased significantly, suggesting a posttranscriptional effect of morphine. In the central amygdala, chronic treatment with morphine significantly increased RNA levels for the NR1 subunit but had no effect on protein levels of any of the 3 subunits, indicating that morphine causes region- and subunit-specific changes in NMDA receptors. Results of bis(sulfosuccinimidyl)suberate cross-linking studies of NR2A subunits suggest that chronic morphine treatment may alter membrane expression (e.g., trafficking or internalization) of this subunit.

In our previous studies, brief ethanol treatment increased the amplitude of GABAergic inhibitory postsynaptic potentials (IPSPs) and diminished glutamatergic EPSPs, indicating a reciprocal alteration of GABAergic and glutamatergic systems. Quantal analysis of spontaneous miniature IPSPs by M. Roberto, Committee on Neurobiology of Addictive Disorders, and microdialysis studies in collaboration with L.H. Parsons, Committee on Neurobiology of Addictive Disorders, indicated that the action of ethanol on IPSPs is predominantly presynaptic, enhancing vesicular GABA release.

Corticotropin-releasing factor (CRF), a neuropeptide most likely involved in stress-induced alcohol drinking, also presynaptically augmented IPSPs in the central amygdala of mice and rats. CRF₁ receptor antagonists and a mutation that deleted the gene for the CRF₁ receptor abolished the effects of both CRF and ethanol, indicating that activation of endogenous CRF₁ receptors mediates the effects of ethanol. The CRF augmentation of IPSPs increased after chronic ethanol treatment, suggesting that a novel synaptic neuroadaptation underlies ethanol dependence.

In collaborative studies with S. Moore, Duke University, we found that ethanol increases vesicular GABA release in neurons in the central amygdala in mice with null mutations in δ opioid receptors significantly more than in those of control mice. Further, ethanol augments vesicular GABA release more after δ opioid receptors are pharmacologically blocked, and agonists for δ opioid receptors diminish IPSPs, indicating that endogenous opioids, like nociceptin, act opposite to the actions of CRF, presynaptically dampening the effects of ethanol on GABAergic synapses.

A μ opioid receptor agonist also diminished IPSPs in neurons in the central amygdala, and null mutations of μ opioid receptors enhanced baseline GABA release of neurons in the central amygdala. However, ethanol significantly increased GABA release in the central amygdala to a comparable extent in both wild-type mice and mice with the null mutation for μ opioid receptors. The subtle differences between the effect of activation of μ and δ opioid receptors on IPSPs in the central amygdala may underlie the behavioral dissimilarities between mice that lack δ opioid receptors and mice that lack μ opioid receptors, in terms of anxiety and ethanol self-administration.

We previously reported that brief ethanol treatment reduced glutamatergic transmission in the central amygdala, in part postsynaptically. However, chronic ethanol treatment and withdrawal increased glutamate release and the depressant effect of ethanol on NMDA-EPSPs, suggesting presynaptic and postsynaptic mechanisms of sensitization to ethanol. Because NR2B mRNA and protein levels and responses to an NR2B-selective antagonist all increased, the postsynaptic effect of chronic ethanol treatment may involve recombination of NMDA receptors in the central amygdala to a preponderance of NR2B subunits. This change in NMDA receptors by chronic ethanol treatment represents another cellular neuroadaptation that underlies ethanol dependence.

In collaboration with T. Bartfai, Molecular and Integrative Neurosciences Department, we have initiated studies on the effects of the neuropeptide galanin on neurons of the dorsal raphe nucleus and the central amygdala, areas that contain galanin and its receptors. We have found that galanin decreases the size of evoked IPSPs in the dorsal raphe nucleus, probably via a presynaptic decrease in GABA release, but augments evoked IPSPs in neurons of the central amygdala. The role of these effects in anxiety and depression are being examined in behavioral studies by Dr. Bartfai and A.J.

Roberts, Molecular and Integrative Neurosciences Department. These combined studies have suggested a new hypothesis of the cellular underpinnings of anxiogenesis and stress-related alcoholism, based on disfacilitation of a disynaptic GABAergic neuronal pathway projecting from the central amygdala to downstream target areas.

PUBLICATIONS

Kang-Park, M.-H., Kieffer, B., Roberts, A.J., Siggins, G.R., Moore, S.D. Presynaptic δ opioid receptors regulate ethanol actions in central amygdala. *J. Pharmacol. Exp. Ther.* 320:917, 2007.

Sharkey, L., Madamba, S.G., Siggins, G.R., Bartfai, T. Galanin alters GABAergic neurotransmission in the dorsal raphe nucleus. *Neurochem. Res.*, *in press*.

Neurobiology of Addiction

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We focus on neural systems that mediate the addictive actions of drugs of abuse. Our emphasis is on understanding the neurobiological basis of vulnerability to relapse and identifying potential treatment targets to prevent relapse.

HYPOTHALAMIC NEUROPEPTIDES AND THEIR THALAMIC PROJECTIONS IN DRUG ADDICTION

The neuropeptides orexin/hypocretin and cocaine- and amphetamine-regulated transcript (CART) are widely distributed throughout the hypothalamus and have been implicated in physiologic and motivational processes related to feeding, energy homeostasis, and arousal. Recent findings suggest that these neuropeptides also play a role in addiction-relevant effects of opiates and psychostimulants. Neurons in the lateral part of the hypothalamus that express orexin/hypocretin are thought to participate in controlling approach behavior (i.e., responses that reflect craving and relapse) motivated by drugs of abuse. CART appears to have a role in modulating the reinforcing effects of cocaine and amphetamine. One of the projection regions of both orexin/hypocretin and CART fibers is the paraventricular thalamus, which, in turn, projects to the ventral and dorsal striatum, brain regions with key roles in motivation, reward, and rein-

forcement. Thus, the paraventricular thalamus is in a strategic anatomic position to integrate peptide signals from hypothalamic afferents and modulate reward-relevant output to the striatum.

We have investigated the role of hypothalamic orexin/hypocretin and CART neurons and their thalamic projection target in drug-seeking behavior by using an animal model of relapse in which drug-directed behavior is elicited by presenting rats with contextual stimuli previously associated with the rewarding effect of cocaine or alcohol. These stimuli elicit drug-seeking behavior in the absence of further drug availability, with effects that persist up to a year. Rats exposed to an alcohol cue that produced strong drug seeking had marked activation of orexin/hypocretin neurons within distinct hypothalamic nuclei and activation of CART neurons in the arcuate nucleus as indicated by brain sections dually labeled for Fos-protein (a marker of neural action) and CART or orexin/hypocretin.

As we had hypothesized, these animals also had substantial activation of neurons within the paraventricular thalamus. These thalamic neurons appeared closely surrounded by a dense plexus of CART- and orexin/hypocretin-immunoreactive terminals as determined from confocal images of triple-labeled brain tissue (Fig. 1). Activation of the paraventricular thalamus, and its projections to the ventral and dorsal striatum, by orexin/hypocretin and CART therefore may be an important mechanism mediating the conditioned incentive effects of drugs of abuse.

We next asked whether the recruitment of hypothalamic orexin/hypocretin neurons is specific to drug seeking or is a neural response associated with appetitively motivated behavior in general. We found that exposure to a cocaine-predictive stimulus significantly increased the number of Fos-positive (i.e., activated) hypothalamic orexin/hypocretin neurons, whereas a stimulus conditioned to sweetened condensed milk (a highly potent natural reinforcer in rats) did not. The apparent specificity of orexin/hypocretin neuronal activation for drug-related events was further confirmed by the finding that a specific antagonist of the orexin-A/hypocretin-1 receptor dose dependently antagonized drug seeking induced by the cocaine-predictive stimulus but did not interfere with behavior induced by a stimulus conditioned to availability of the natural reinforcer.

These findings confirm an addiction-relevant role for hypothalamic orexin/hypocretin neurons, identify

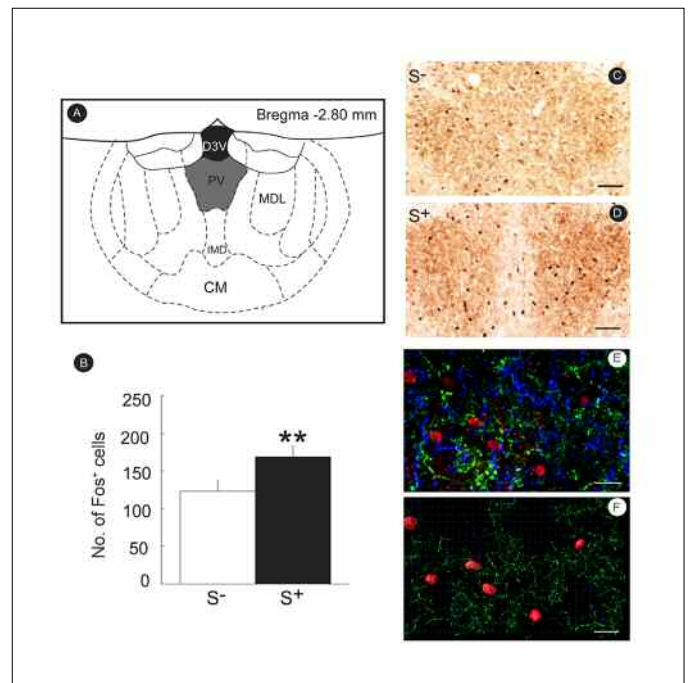


Fig. 1. A, Rostrocaudal level at which photomicrographs in C and D were obtained. B, Number of Fos-positive cells in the paraventricular thalamus induced by presentation of an alcohol-associated environmental stimulus (S⁺) or a neutral stimulus (S⁻). C and D, Effects of exposure to S⁺ and S⁻ on the number of Fos-positive cells in the paraventricular thalamus. Scale bar = 50 μ m. E, Confocal image of triple-labeled tissue from the paraventricular thalamus shows close association between orexin/hypocretin (blue) and CART (green) immunoreactive terminals and Fos-positive (red) nuclei. E, Three-dimensional image reconstructed from a raw confocal image confirms the association of Fos-positive nuclei with orexin/hypocretin and CART fibers. Scale bar in E and F = 10 μ m. Reprinted with permission from Dayas, C.V., McGranahan, T.M., Martin-Fardon, R., Weiss, F. Stimuli linked to ethanol availability activate hypothalamic CART and orexin neurons in a reinstatement model of relapse. *Biol. Psychiatry*, *in press*. Copyright Elsevier 2007.

CART neurons in the arcuate nucleus as a second hypothalamic signal involved in drug seeking and craving, and implicate the paraventricular thalamus as an important site for the integration of reward-relevant orexin/hypocretin- and CART-coded hypothalamic input. Moreover, the responsiveness of orexin/hypocretin neurons to stimuli associated with cocaine and alcohol but not natural reward and the selective reversal of drug seeking but not behavior directed at obtaining natural reward by a orexin-A/hypocretin-1 receptor antagonist indicate that the orexin/hypocretin system is an important target for studying the neural control of maladaptive drug seeking as opposed to normal motivated behavior. Last, these hypothalamic neuropeptide systems may be important targets for the design of pharmacotherapies to treat addiction.

POTENTIAL PHARMACOLOGIC TARGETS FOR TREATMENT OF COCAINE WITHDRAWAL AND PREVENTION OF RELAPSE

Cocaine withdrawal is often associated with anxiety and increased stress reactivity, conditions implicated clinically as major risk factors for relapse. We have focused on 2 receptor systems with a possible role in these symptoms of cocaine withdrawal: cannabinoid-1 (CB₁) and metabotropic glutamate (mGlu) receptors. Activation of CB₁ receptors can attenuate anxiogenic effects often associated with cocaine administration. We determined whether daily treatment with a CB₁ receptor agonist during a 2-week cocaine withdrawal period modified anxiety-like and drug-seeking behavior in rats. The major finding was that a low dose of the agonist reduced cocaine seeking in an animal model of relapse and anxiolytic-like effects in an animal model of anxiety. A high dose produced the opposite effects, increasing anxiety and cocaine seeking. Previously, we had identified mGlu2/3 receptors as novel regulatory mechanisms for withdrawal-associated anxiety and hyperresponsiveness to stress. More recently, we found a similar possible role for the mGlu5 receptor. An mGlu5 antagonist dose dependently reversed cocaine seeking in an animal model of stress-induced relapse.

Our results implicate both endocannabinoid and metabotropic glutamate receptors in anxiety and stress-like symptoms of cocaine withdrawal. Moreover, the results indicate the CB₁ receptors as well as mGlu2/3 and mGlu5 receptors are potential pharmacologic targets for withdrawal-related anxiety and prevention of relapse.

PUBLICATIONS

Dayas, C.V., Liu, X., Simms, J.A., Weiss, F. Distinct patterns of neural activation associated with ethanol-seeking: effects of naltrexone. *Biol. Psychiatry* 61:979, 2007.

Dayas, C.V., McGranahan, T.M., Martin-Fardon, R., Weiss, F. Stimuli linked to ethanol availability activate hypothalamic CART and orexin neurons in a reinstatement model of relapse. *Biol. Psychiatry*, *in press*.

Economidou, D., Fedeli, A., Martin-Fardin, F.R., Weiss, F., Massi, M., Ciccioppo, R. Effect of novel nociceptin/orphanin FQ-NOP receptor ligands on ethanol drinking in alcohol-preferring msP rats. *Peptides* 27:3299, 2006.

González-Cuevas, G., Aujla, H., Martin-Fardon, F., López-Merono, J.A., Navarro, M., Weiss, F. Subchronic cannabinoid agonist (WIN 55,212-2) treatment during cocaine abstinence alters subsequent cocaine seeking behavior. *Neuropsychopharmacology*, *in press*.

Martin-Fardon, F.R., Maurice, T., Aujla, H., Bowen, W.D., Weiss, F. Differential effects of σ_1 receptor blockade on self-administration and conditioned reinstatement motivated by cocaine vs natural reward. *Neuropsychopharmacology* 32:1967, 2007.

Galanin Receptor Ligands in the Treatment of Anxiety and Depression

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The past year brought several breakthroughs in defining the galanin 1 receptors as drug targets for the treatment of anxiety disorders and major depression. In rodent models of antidepressant efficacy, galnon, a galanin receptor agonist, produced an antidepressant-like effect similar to that of fluoxetine and imipramine. Many antidepressants also promote neurogenesis, and we found that the type 2 galanin receptor mediates effects that promote neuroprotection or neurogenesis in the hippocampus. These data suggest that galanin receptor 2 is a putative drug target for a new class of antidepressant drugs. Because novel drug targets for major diseases are rare, this important breakthrough has captured the interest of both the academic and the industrial sectors.

Using compounds synthesized by J. Rebeck, Jr., the Skaggs Institute for Chemical Biology, we and scientists in Denmark have found that galanin receptor 3 is also a target of anxiolytic and antidepressant actions. In collaboration with Dr. Rebeck and E. Roberts, Department of Chemistry, we are searching for new chemotypes of galanin type 3 receptors to test in models of anxiety and depression.

Thermoregulation Mechanisms: Thermosensitivity in the Brain

I.V. Tabarean, B. Conti, M. Sánchez-Alavez, C. Davis, H. Korn, T. Bartfai

Thermosensitivity of some neurons in the anterior hypothalamus underlies the regulation of core body temperature, fever response, and energy metabolism, yet little is known about these important, but rare, cells. Using cell cultures of primary neurons and slice preparations, we have shown that individual neurons without the presence of a neuronal network can sense cold and warm temperatures and can change firing rate in response to these temperature changes.

Thus, warm sensitivity is an intrinsic property of these neurons and not a network property. The warm-sensitive neurons express receptors for several pyrogenic agents, such as prostaglandin E₂, IL-1, and calcitonin gene-regulated peptide, which are involved in mediating fever in response to inflammation and infection and in the generation of hot flashes in women after menopause and in men receiving endocrine therapy for prostate cancer.

We also found that adenosine and histamine reduce thermosensitivity, and we are defining the receptor subtypes through which these effects are exerted. A molecular and cellular understanding of the central temperature set point is a prerequisite for new treatments of feeding and sleep disorders because these phenomena are closely coordinated with and depend mutually on changes in the set point. Recent interest in the role of these neurons in regulating "burn rate" in obesity has highlighted them as targets for drug therapies.

Inflammation and Obesity

M. Sánchez-Alavez, B. Conti, T. Bartfai

Recognition is increasing that obesity is associated with low-grade inflammation and that inflammatory mediators are involved in the progression from obesity to insulin resistance and to type 2 diabetes. Using mice provided by S. Narumiya, Kyoto University, Kyoto, Japan, that lack EP3 prostanoid receptors, we found that the animals not only have a defective fever response but also have early-onset obesity with insulin resistance and glucose tolerance. The association between impairment of prostaglandin signaling, which is involved in inflammation, and obesity is made even more interesting by the observations that these animals have a night eating/binging behavior similar to the behavior of some humans with obesity.

PUBLICATIONS

Barr, A.M., Kinney, J.W., Hill, M.N., Lu, X., Biros, S., Rebek, J., Jr., Bartfai, T. A novel, systemically active, selective galanin receptor type-3 ligand exhibits antidepressant-like activity in preclinical tests. *Neurosci. Lett.* 405:111, 2006.

Bartfai, T. From lab bench to Wall Street. *FASEB J.* 10:1578, 2006.

Bartfai, T., Lees, G.V. Drug Discovery: From Bedside to Wall Street. Academic Press, San Diego, 2006.

Conti, B., Maier, R., Barr, A.M., Morale, M.C., Lu, X., Sanna, P.P., Bilbe, G., Hoyer, D., Bartfai, T. Region-specific transcriptional changes following the three antidepressant treatments: electroconvulsive therapy, sleep deprivation and fluoxetine. *Mol. Psychiatry* 12:167, 2007.

Conti, B., Sánchez-Alavez, M., Winsky-Sommerer, R., Morale, M.C., Lucero, J., Brownell, S., Fabre, V., Huitrón-Reséndiz, S., Henriksen, S., Zorrilla, E.P., de Lecea, L., Bartfai, T. Transgenic mice with a reduced core body temperature have an increased life span. *Science* 314:825, 2006.

Kovacs, Z., Kekesi, K.A., Szilagy, N., Abraham, I., Szekacs, D., Kiraly, N., Papp, E., Csaszar, I., Szego, E., Barabas, K., Peterfy, H., Erdei, A., Bartfai, T., Juhász, G. Facilitation of spike-wave discharge activity by lipopolysaccharides in Wistar Albino Glaxo/Rijswijk rats. *Neuroscience* 140:731, 2006.

Robinson, J.K., Bartfai, T., Langel, Ü. Galanin/GALP receptors and CNS homeostatic processes. *CNS Neurol. Disord. Drug Targets* 5:327, 2006.

Sánchez-Alavez, M., Klein, I., Brownell, S.E., Tabarean, I.V., Davis, C.N., Conti, B., Bartfai, T. Night eating and obesity in the EP3R-deficient mouse [published correction appears in *Proc. Natl. Acad. Sci. U. S. A.* 104:9911, 2007]. *Proc. Natl. Acad. Sci. U. S. A.* 104:3009, 2007.

Sollenberg, U.E., Lundström, L., Bartfai, T., Langel, Ü. M871: a novel peptide antagonist selectively recognizing the galanin receptor type 2. *Int. J. Pep. Res. Ther.* 12:115, 2006.

Tabarean, I.V., Korn, H., Bartfai, T. Interleukin-1 β induces hyperpolarization and modulates synaptic inhibition in preoptic and anterior hypothalamic neurons. *Neuroscience* 141:1685, 2006.

Zorrilla, E.P., Brennan, M., Sabino, V., Lu, X., Bartfai, T. Galanin type 1 receptor knockout mice show altered responses to high-fat diet and glucose challenge. *Physiol. Behav.* 91:479, 2007.

Hypothalamic Regulated Homeostasis in Health and Disease

B. Conti, H. Bajova, R. Fernandez, S. Alboni, M. Sánchez-Alavez

Our areas of interest include hypothalamic regulation of (1) temperature/energy homeostasis and (2) immune functions. We recently developed transgenic mice with constitutive lowered core body temperature (CBT) and prolonged life span that are being used to study the mechanisms of aging and energy homeostasis. In our research in neuroimmunology, we focus on the role of the stress-induced proinflammatory cytokine IL-18 in health and disease. IL-18 is used in studies of the correlation between stress and susceptibility to or progression of diseases.

REGULATION OF CORE BODY TEMPERATURE IN AGING AND ENERGY HOMEOSTASIS

Reduction of CBT has antiaging effects and prolongs life span in poikilotherms. In homeotherms, a lowered CBT is associated with calorie restriction, a controlled dietary regimen that prolongs life span in rodents and monkeys and delays the progression of a variety of diseases. Researchers have proposed that a reduction of CBT per se could contribute to the antiaging

ing effects of calorie restriction. To test this hypothesis, we generated transgenic mice with a reduced CBT.

We hypothesized that local heat production in the vicinity of the "central thermostat" located in the preoptic area of the hypothalamus could mimic an increase in CBT and activate thermoregulatory compensatory mechanisms that ultimately result in a reduction of CBT. To achieve this goal, we overexpressed the uncoupling protein 2 (UCP2) exclusively in neurons that express hypocretin (Hcrt-UCP2 mice). UCP2 is an inner mitochondrial membrane protein that uncouples oxidative phosphorylation from respiration, dissipating the proton-gradient energy in the form of heat. Hypocretin neurons are uniquely found in the lateral part of the hypothalamus 0.8 mm from the preoptic area.

Hcrt-UCP2 mice have increased temperature elevation in the lateral part of the hypothalamus and in the preoptic area, resulting in a modest (0.3°C–0.6°C) but long-term reduction of CBT. When fed ad libitum, Hcrt-UCP2 mice have a calorie intake similar to that of their wild-type littermates but have a 17%–19% increase in life span. Thus, a small but prolonged reduction of CBT resulted in an increased life span independent of altered diet or calorie restriction. Analysis of the mortality rate indicated that aging of Hcrt-UCP2 mice is similar to that of calorie-restricted mice. Furthermore, Hcrt-UCP2 mice have an increased metabolic efficiency and, like calorie-restricted mice, have an age-dependent reduction of markers of oxidative stress. This finding suggests that a long-term reduction of CBT may influence the formation of free radicals and slow the accumulation of age-related damage. In ongoing research, we are determining the mechanisms that mediate the prolonged life expectancy in these mice and the implications of reduced CBT in energy homeostasis.

IL-18 AS AN IMMUNE, NEURONAL, AND ENDOCRINE SIGNAL

IL-18 is a pleiotropic cytokine that acts as an immune, neuronal, and endocrine signal. IL-18 can stimulate both the cellular and the humoral immune response, influencing a great variety of diseases, including tumor growth; infections; progression of autoimmune diseases such as diabetes, multiple sclerosis, and rheumatoid arthritis; and atherosclerosis.

Previously, we cloned rat IL-18 and started characterizing its role in the CNS and as a CNS-mediated modulator of immune functions. We showed that after neurogenic stimulation, stress caused by restraint, or treatment with corticotropin, the level of IL-18 is ele-

vated in a tissue-specific manner via differential use of promoters in the adrenal cortex, immunocompetent cells, the pituitary gland, and the neurons of the habenula. We are examining the regulation of IL-18 induction during stress at transcriptional and posttranscriptional levels to identify which CNS and adrenal signals, including corticotropin and glucocorticoids, can affect the production of the cytokine. The results will improve our understanding of the mechanisms by which stress, psychological factors, and emotional states can influence the etiology and the progression of diseases such as atherosclerosis and autoimmunity. We are also investigating the role of stress-induced IL-18 in atherosclerosis and in the activation of microglia.

PUBLICATIONS

Conti, B., Sánchez-Alavez, M., Winsky-Sommerer, R., Morale, M.C., Brownell, S., Fabre, V., Huitrón-Reséndiz, S., Henriksen, S., Zorrilla, E.P., de Lecea, L., Bartfai, T. Transgenic mice with reduced core body temperature have an increased life span. *Science* 314:825, 2006.

Sugama, S., Fujita, M., Hashimoto, M., Conti, B. Stress-induced morphological microglial activation in the rodent brain: involvement of interleukin-18. *Neuroscience* 146:1388, 2007.

IL-1 β Modulation of Synaptic Inhibition in Preoptic and Anterior Hypothalamic Neurons

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The proinflammatory cytokine IL-1 β affects neuronal activity under both physiologic and pathophysiologic conditions. The molecular mechanism of the rapid actions of IL-1 β in neurons is not known. Some of the effects of the cytokine are mediated by induction of cyclooxygenase-2 and the subsequent synthesis and release of prostaglandin E₂. This process takes 30–60 minutes, but IL-1 β also exerts faster neuronal actions in the preoptic area and anterior part of the hypothalamus. Using whole-cell patch-clamp recordings, we have studied the fast (1–3 minutes) signaling by IL-1 β in preoptic and anterior hypothalamic neurons. Exposure to IL-1 β hyperpolarized a subset of neurons, decreased their input resistance, and reduced their firing rate. The thermosensitivity of the neurons decreased in response to the cytokine. These effects were associated with an increased frequency of bicuculline-sensitive spontaneous inhibitory postsynaptic

currents and miniature inhibitory postsynaptic currents, indicating a presynaptic mechanism of action.

The effects of IL-1 β require the type 1 IL-1 receptor (IL-1R1) and the adapter protein myeloid differentiation primary response protein (MyD88); the cytokine was ineffective in cultures obtained from mice lacking the gene for IL-1R1 or for MyD88. In addition, we found that the second messenger ceramide, produced by activation of the neutral sphingomyelinase by IL-1R1–MyD88, also increased the frequency of miniature inhibitory postsynaptic currents. Both IL-1 β and ceramide reduced the A-type potassium currents in preoptic and anterior hypothalamic neurons. This reduction accounts for the increased spontaneous inhibitory postsynaptic current frequency. Our results suggest that IL-1 β inhibits the activity of preoptic and anterior hypothalamic neurons by increasing the presynaptic release of γ -aminobutyric acid.

PUBLICATIONS

Tabarean, I.V., Korn H., Bartfai, T. Interleukin-1 β induces hyperpolarization and modulates synaptic inhibition in preoptic and anterior hypothalamic neurons. *Neuroscience* 141:1685, 2006.

Sánchez-Alavez, M., Tabarean, I.V., Behrens, M.M., Bartfai, T. Ceramide mediates the rapid phase of febrile response to interleukin-1 β . *Proc. Natl. Acad. Sci. U. S. A.* 103:2904, 2006.