Instituto Juan March
de Estudios e Investigaciones

135 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Molecular Mechanisms of Immune Modulation: Lessons from Viruses

Organized by

A. Alcami, U. H. Koszinowski and M. Del Val

A. Alcami
C. Boshoff
M. Del Val
M. Esteban
P. Ghazal
H. Hengartner
D. C. Johnson
U. H. Koszinowski
S. A. Lira
C. Martínez-A.
G. McFadden
A. J. McMichael
E. S. Mocarski
B. Moss
A. A. Nash
H. L. Ploegh
A. A. Scalzo
G. L. Smith
J. W. Yewdell

Instituto Juan March
de Estudios e Investigaciones

135 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Molecular Mechanisms of Immune Modulation: Lessons from Viruses

Organized by

A. Alcami, U. H. Koszinowski and M. Del Val

A. Alcami
C. Boshoff
M. Del Val
M. Esteban
P. Ghazal
H. Hengartner
D. C. Johnson
U. H. Koszinowski
S. A. Lira
C. Martínez-A.
G. McFadden
A. J. McMichael
E. S. Mocarski
B. Moss
A. A. Nash
H. L. Ploegh
A. A. Scalzo
G. L. Smith
J. W. Yewdell

The lectures summarized in this publication were presented by their authors at a workshop held on the 25h through the 27th of February 2002, at the Instituto Juan March.

Depósito legal: M-15.857/2002
Impresión: Ediciones Peninsular. Tomelloso, 27. 28026 Madrid.

INDEX

Introduction

A. Alcami, U. H. Koszinowski
and M. Del Val

Viruses have evolved strategies to evade the powerful host inflammatory and immune responses that can eliminate them. This was the subject of a previous Juan March Institute Workshop on 'Viral evasion of host defense mechanisms' organised by M. B. Mathews and M. Esteban in September 1993 (booklet n° 19). The number of viral immune evasion strategies identified has increased dramatically in recent years. Large DNA viruses (herpesviruses and poxviruses) have the capacity to encode many proteins that mimic or target specific components of the host immune system, such as homologues of cytokines and chemokines, and their receptors, or proteins that target specific components of the antigen presentation pathways and prevent immune recognition. The function of these viral molecules is to evade immune responses or to promote viral replication, and they may also contribute to pathology. We have just started to understand the immune evasion strategies at the molecular level, and the list of these strategies forms the 'Who is who' of today's immunology.

This workshop brought together scientists with different views of the interaction of viruses with host defence mechanisms. The aim of the meeting was to discuss strategies of immune modulation encoded by viruses, and focused on large DNA viruses (herpesviruses and poxviruses) and other viruses such as human immunodeficiency virus (HIV). A number of models for viral pathogenesis were discussed since these are helping us to understand the function of different arms of the immune system in anti-viral defence *in vivo*. The use of animal models of viral infection is starting to uncover the physiological role of viral immunomodulatory molecules and is an area of research that is becoming very active.

The emphasis of the meeting was to discus how these studies can teach us about the host immune system. These viral proteins have been optimized for millions of years of evolution as effective immunomodulatory molecules and some of them have sequence similarity to human proteins. We can now use the information found in viral genomes to uncover new components of the immune system, to understand cellular processes involved in protein trafficking and antigen presentation, and to identify novel mechanisms of immune modulation. These findings will help us to treat virus-induced pathology and to design safer and more immunogenic vaccines, and will lead to new strategies of therapeutic intervention in human diseases that are caused by an over-reactive immune and inflammatory response.

A. Alcami, U. H. Koszinowski and M. Del Val

Session 1: Immune response to viral infection
Chair: Ulrich H. Koszinowski

Humoral and cell mediated immunity in antiviral protection

Hans Hengartner

Experimental Immunology, University and ETH Zürich,
Schmelzbergstr. 12, CH-8091 Zurich

The nature of the virus and the genetic background of the infected organism are important factors which determine the type of an antiviral immune response. Degree of cytopathogenicity, tropism, virulence or persistence are decisive which arm of the immune system will be triggered and which effector mechanisms will contribute to control a virus infection efficiently. As model systems for virus infections in the mouse for a typical non-cytopathic virus, we used the lymphocytic choreomeningitis virus (LCMV) and for a cytopathic virus the vesicular stomatitis virus (VSV).

An efficient control of VSV infection depends on the early appearance of IgM and IgG antibodies. Antibodies at day 3 to 4 post infection were devoid of somatic mutations in their variable regions and exhibited binding avidities of up to 10^9 M^{-1}. Antibodies of the secondary immune response type, however, do contain somatic mutations avidities up to 9 x 10^9 M^{-1}. The surprising finding was that antibody mediated protection was dependent on a minimal antibody avidity threshold of around 0.5-1 x 10^8 M^{-1} and a minimum serum antibody concentration of 1 to 10 μg/ml.

The early control of the non-cytopathic LCMV infection depends on efficient induction of cytotoxic T lymphocytes exhibiting cytotoxic activity or secretion capacity of non-cytopathic antiviral mediators. In addition to these early cytotoxic T lymphocyte responses, neutralising antibodies contribute substantially to the longterm immune control of non-cytopathic viruses as will be shown for the infection with LCMV. The high virus load during the initial phase of an infection and the ability of this virus to spontaneously acquire mutations are important prerequisites for escaping an ongoing immune response. In this context, LCMV escape from the humoral immune response by single point mutations in neutralising envelope protein determinants may occur, particularly during conditions of CTL deficiency leading to virus persistence and loss of $CD4^+$ T cell responsiveness. Similar mechanisms might contribute to the persistence of human pathogens such as hepatitis B and HI viruses.

References:

1. Kalinke, U., A. Oxenius, C. Lopez-Macias, R. M. Zinkernagel, and H. Hengartner. 2000. Virus neutralization by germ-line vs. hypermutated antibodies. *Proc.Natl.Acad.Sci U.S.A* 97:10126-10131.

2. Bachmann, M. F., U. Kalinke, A. Althage, G. Freer, C. Burkhart, H. Roost, M. Aguet, H. Hengartner, and R. M. Zinkernagel. 1997. The role of antibody concentration and avidity in antiviral protection. *Science* 276:2024-2027.

3. Ciurea, A., P. Klenerman, L. Hunziker, E. Horvath, B. M. Senn, A. F. Ochsenbein, H. Hengartner, and R. M. Zinkernagel. 2000. Viral persistence in vivo through selection of neutralizing antibody-escape variants. *Proc.Natl.Acad.Sci U.S.A* 97:2749-2754.

4. Ciurea, A., L. Hunziker, P. Klenerman, H. Hengartner, and R. M. Zinkernagel. 2001. Impairment of CD4+ T cell responses during chronic virus infection prevents neutralizing antibody responses against virus escape mutants. *J.Exp.Med.* 193:297-306.

Mechanisms of gammaherpesvirus induced pathology in the lymphoid system

A A Nash

Department of Veterinary Pathology, University of Edinburgh

Gammaherpesvirues have been found in a variety of animal species and are associated with important diseases in man (EBV, KSHV) and livestock (OHV-2, AlHV associated with Malignant Catarrhal Fever). A feature of gammaherpesvirus infection is the establishment of latency in lymphocytes and the association with lymphoproliferative disorders. These disorders range from lymphoproliferation associated with splenomegaly (similar to infectious mononucleosis) to the generation of B cell lymphomas. To study the role of the virus and the immune system in pathology of the lymphoid system we have been investigating the properties of a murine gammaherpesvirus, MHV-68.

This virus infects epithelial and mononuclear cells in the respiratory tract. A productive infection is observed for around 10days and is controlled by the immune system notably CD8 T cells. The virus establishes a latent/persistent infection in B cells, macrophages and lung epithelial cells. B cells in the draining lymph node and the spleen undergo proliferation resulting in lymphadenopathy and splenomegaly. This lymphoproliferation is driven by CD4 T cells and is dependent on the activity of IFN-γ. In mice deficient in IFN-γ receptor (IFN-γR-/-), splenomegaly fails to occur resulting in splenic atrophy.

Atrophy is the result of a dramatic loss of lymphocytes from the spleen and lymph node, disorganisation of lymphoid architecture and the deposition of lagre amounts of collagen, resulting in fibrosis. Cytokine levels in the atrophied spleen show an enhanced production of the cytokines IFN-γ, TNF-α, IL-1β, TGF-β and greatly reduced levels of the chemokines IP-10 and MIG, important in the recruitment of activated T cells to sites of infection. The peak pathology is seen 2 to 3 weeks post infection and intriguingly the spleen and draining lymph node begin to resolve the fibrosis, leading to a repopulation of the affected tissues.

Although there is a dramatic reduction in the number of CD4 and CD8 T cells during the peak of splenic atrophy, this pathology is dependent on the activity of CD8 T cells. In IFN-γR-/- mice deficient in CD8 T cells splenic atrophy does not occur, instead there is a vigorous splenomegaly.

The MHV-68 genes associated with splenomegaly and splenic atrophy are linked, in part, to the left end of the viral genome. This region encodes four novel genes, M1 to M4 and eight tRNA-like genes. Only M3 has so far been characterised as a broad-spectrum chemokine binding protein, which is thought to delay migration of inflammatory cells and T cells to sites of infection. Using another strain of murine gammaherpesvirus, MHV-76, which lacks M1 to M4 and the vtRNA's but is otherwise identical to MHV-68, we showed that that this virus failed to induce a significant splenomegaly and also failed to trigger splenic atrophy in IFN-γR-/- mice. Using other mutants we have begun to map regions in the left end important in mediating both forms of lymphoid pathology.

References:

Nash, AA, Dutia, BM, Davison, AJ and Stewart, JP. (2000). Natural history of murine gamma-herpesvirus infection. *Phil.Trans.R.Soc Lond.* B **356**: 569-579.

Dutia, BM, Clarke, CJ, Allen, DJ and Nash,AA. (1997). Pathological changes in the spleens of gamma interferon receptor-deficient mice infected with murine gammaherpesvirus: a role for CD8 T cells. *J. Virol.* **71**: 4278-4283.

Ebrahimi, B, Dutia,BM, Brownstein, DG and Nash, AA. (2001). Murine gammaherpesvirus-68 infection causes multi-organ fibrosis and alters leucocyte trafficking in interferon-γ receptor knockout mice. *Am. J Path.* **158**:2117-2125.

KSHV and the host: A molecular arms race

Justin Stebbing, Dimitra Bourmpoulia, John Wilkinson, Frances Gotch and Chris Boshoff

Wolfson Institute for Biomedical Research, Cruciform Building, Gower Street
University College London

KSHV encodes an array of cellular homologues that appear to be involved in anti-host immune responses. These include at least 3 chemokines, 4 interferon regulatory factor-like (IRF's) proteins and a FLIP (FLICE-inhibitory protein) homologue. In addition, KSHV encodes 2 proteins (K3 and K5) that interfere with MHC class I presentation; human genome database searches indicate that K3 and K5 both have cellular homologues, although their functions have not yet been elucidated. The introduction of Highly Active Anti-retroviral Therapy (HAART) has led to a decline in the incidence of Kaposi's sarcoma (KS) in the West, and to the resolution of established lesions. CTL activity against KSHV proteins has been demonstrated, however identification of CTL epitopes has been elusive and problematic.

Although herpesvirus genomic layout is relatively conserved, KSHV encodes a hypervariable oncoprotein K1, which is under more intense selection pressure than any other known viral or mammalian gene. Using patient specific (autologous) overlapping peptides for K1, we have demonstrated multiple functional human leukocyte antigen (HLA) restricted CTL epitopes at sites of positive selection within K1 and used these to monitor immunological responses in HIV-1/KSHV co-infected individuals. We discovered KSHV intra-strain conservation of immunogenic CTL epitopes, but inter-strain antigenic variation and diversity. The implications in terms of the viral-host equilibrium and the development of strain-specific vaccines will be discussed.

Regulatory loops in primary and latent viral infections

Peter Ghazal

The relationship between the immune system, infected cells and latent viruses are highly complex and dynamic. Latent viruses not only avoid elimination by the host's primary immune response, they also remain for life in the presence of strong acquired immunity, quite often exhibiting frequent periods of reactivation. This talk will explore mechanisms of virus-host homeostasis using genomic approaches. The role of immune interference strategies by viruses in establishing a key regulatory loop in latency will be highlighted. Furthermore, the significance of cellular heterogeneity on viral activity and, the concept of threshold limits in naive and memory states of immunity to account for the failure of the host to completely eradicate an infection will be discussed.

Rerefences:

Ghazal, P., J.C. González-Armas, J.J. García-Ramírez, S. Kurz and A. Angulo. Viruses: Hostages to the cell. Virol. 275: 233-237, 2000.

Redpath, S., A. Angulo, N.R.J. Gascoigne and P. Ghazal. Immune checkpoints in viral latency. Annu. Rev. Microbiol. 54: In Press 2001.

Session 2: Modulation of cytokine and chemokine responses I
Chair: Antonio Alcami

Immune evasion strategies by myxoma virus

Grant McFadden

Department of Microbiology and Immunology
The University of Western Ontario and The John P. Robarts Research Institute
London, N6G 2V4 Ontario, CANADA

The larger DNA viruses (i.e., the adenoviruses, herpesviruses, iridoviruses and poxviruses) specifically encode proteins that function to protect the virus from immune recognition and/or clearance by the infected host. In the 1980's the term "virokine" was proposed to describe virus-encoded proteins secreted from infected cells which function by mimicking extracellular signaling molecules such as cytokines or other secreted regulators important for the host immune repertoire. Later, in the 1990's the term "viroceptor" was introduced to account for the observation that some virus encoded proteins mimic important cellular receptors and function by diverting host cytokines away from their normal receptors, thus interrupting the immune circuitry at its earliest stages. The interplay between these complementary strategies are exemplified by ongoing studies from our lab on one particular poxvirus, myxoma virus, which is the infectious agent of a virulent systemic disease of domestic rabbits, myxomatosis. Originally described by G. Sanarelli in the last century, myxoma was the first virus pathogen discovered for a laboratory animal and was the first viral agent ever deliberately introduced into the environment for the explicit purpose of pest eradication. Since its release into the Australian and European feral rabbit populations more than 40 years ago, the field strains of both the rabbit and virus have been subjected to mutual evolutionary and selective pressures that have resulted in a steady-state enzootic in the inoculated areas. Myxoma shares many of the biologic features associated with other poxviruses, namely cytoplasmic location of replication and a large double stranded DNA genome (160 kilobases). Multiple lines of evidence indicate that myxoma encodes many gene products whose function is to permit the spread and propagation of the virus in a variety of host tissues. Some of these viral proteins specifically counteract or subvert the development of the host inflammatory response and acquired cellular immunity. Examples of these include virokines such as SERP-1 (a secreted serpin) and viroceptors that target TNF, interferon-γ and chemokines. Recent work implicating virus pathogenesis with the host chemokine network will be highlighted.

Poxvirus inhibitors of chemokines and cytokines

Geoffrey L. Smith

The Wright-Fleming Institute, Faculty of Medicine, Imperial College, London

Cytokines and chemokines play an important role in the immune response to infection and consequently evolution has selected viruses that have developed measures to counteract the action of these host proteins. Large DNA viruses in particular display a wide variety of defensive strategies.

To combat chemokines, some viruses, such as members of the herpes and poxvirus families, express cytokines that bind to chemokine receptors on host cells but do not induce signal transduction by these receptors and thus act as antagonists of the host chemokines. Other chemokines expressed by some herpesviruses are able to both bind and signal via specific chemokine receptors. The specificity of this signalling is presumably beneficial to the virus in the host animal. Another strategy employed by herpes and poxviruses is to express proteins on the surface of virus-infected cells that are related to the 7-transmembrane chemokine receptors of cells. These molecules might either bind host chemokines and not signal, thereby acting as a sink to soak up host chemokines, or both bind host chemokines and induce signal transduction that is somehow beneficial to virus replication. Lastly, herpes and poxviruses express soluble proteins that are unrelated to proteins from hosts but which bind chemokines in solution. These chemokine-binding proteins may bind the chemokine through either the proteoglycan-binding domain or through the chemokine receptor-binding domain. In the former case, after the chemokine is bound to the virus protein, it may still bind the chemokine receptors on leukocytes and stimulate these cells. However, these cells are less well recruited to areas of inflammation because the proteoglycan-binding domain of the chemokine is masked by the virus protein. In the latter case, the chemokine is unable to bind to its natural receptors and so leukocytes cannot be activated or recruited.

Viruses also display multiple strategies against cytokines and can act to inhibit their action at different stages. Some poxviruses synthesise an intracellular protein that inhibits the enzyme needed for the processing of pro-interleukin (IL)-1β and pro-IL-18 into their mature forms that are secreted from the cell. Other poxvirus proteins are secreted from the cell and bind and inhibit cytokines in solution, for instance tumour necrosis factor, IL-1β, interferon-g, IFN-α/β and IL-18. Proteins that bind IFN-α/β and TNF can also do this on the cell surface. In most cases these virus cytokine binding proteins have amino acid similarity to host cytokine receptors on the cell surface. Other secreted proteins made by herpesviruses and poxviruses are related to cytokines (such as IL-10, IL-6, IL-17 and epidermal or vascular endothelial growth factors) and induce biological responses favourable to the virus life cycle. Lastly, viruses can inhibit the intracellular signalling cascades induced by the binding of cytokines or chemokines to their cell receptors. This inhibition can be mediated at different stages during these signalling cascades.

A review of these virus strategies to modulate cytokine and chemokine activity will be given with emphasis on strategies employed by poxviruses and which have been investigated recently.

References:

Smith, G.L., Symons, J.A., Khanna, A., Vanderplasschen, A. & Alcamí, A. (1997). Vaccinia virus immune evasion. Immunol. Revs. **159**, 137-154.

Alcamí, A., and Koszinowski, U. H. (2000). Viral mechanisms of immune evasion. Trends Microbiol. **8**, 410-418.

Moss, B., & Shisler, J.L. (2001). Immunology 101 at poxvirus U: immune evasion genes. Semin. Immunol. **13**, 59-66.

Immune-defense molecules of Molluscum contagiosum virus: a human poxvirus

Bernard Moss, Joanna L. Shisler, Yan Xiang and Tatiana G. Senkevich

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, 20892-0445, USA

Molluscum contagiosum virus (MCV) and variola virus, the only poxviruses that rely exclusively on humans as hosts, belong to different genera and are only distantly related. Infection with variola virus caused smallpox, an acute infection with a high fatality rate. By contrast, MCV produces small, benign papular skin lesions that can go unnoticed in healthy individuals. MCV has a world-wide distribution and a high percentage of individuals have been infected. The virus-filled lesions can persist for months without signs of inflammation, suggesting local immunosuppression. Nevertheless, the MCV lesions are extensive in immunocompromised individuals including those with AIDS, indicating an important role for host immunity in containing if not rapidly eliminating the infection. Analysis of the MCV genome sequence revealed approximately 100 essential genes common to other vertebrate poxviruses. Unexpectedly, MCV lacks most of the immune defense molecules that have been described in other poxviruses but contains more than 70 open reading frames unique to MCV. The latter include a homolog of a cellular chemokine with antagonistic activity, a major histocompatibility complex (MHC) class I molecule that binds β2-microglobulin, a selenocysteine-containing glutathione peroxidase, an IL-18 binding protein that inhibits interferon-γ induction, and a death effector domain protein that prevents apoptosis.

References:

Senkevich, T. G., J. J. Bugert, J. R. Sisler, E. V. Koonin, G. Darai, and B. Moss 1996. Genome sequence of a human tumorigenic poxvirus: prediction of specific host response-evasion genes. Science 273:813-816.

Shisler, J. L., T. G. Senkevich, M. J. Berry, and B. Moss 1998. Ultraviolet-induced cell death blocked by a selenoprotein from a human dermatotropic poxvirus [see comments]. Science 279:102-105.

Senkevich, T. G., and B. Moss 1998. Domain structure, intracellular trafficking, and beta 2- microglobulin binding of a major histocompatibility complex class I homolog encoded by molluscum contagiosum virus. Virology 250:397-407.

Xiang, Y., and B. Moss 1999. IL-18 binding and inhibition of interferon gamma induction by human poxvirus-encoded proteins. Proc. Natl. Acad. Sci. USA 96:11537-11542.

Luttichau, H. R., J. Stine, T. P. Boesen, A. H. Johnsen, D. Chantry, J. Gerstoft, and T. W. Schwartz 2000. A highly selective CC chemokine receptor (CCR)8 antagonist encoded by the poxvirus molluscum contagiosum. J. Exp. Med. 191:171-179.

Shisler, J. L., and B. Moss 2001. Molluscum contagiosum virus inhibitors of apoptosis: The MC159 v-FLIP protein blocks Fas-induced activation of procaspases and degradation of the related MC160 protein. Virology 282:14-25.

A new type of soluble interferon-gamma receptor encoded by fowlpox virus

Florian Puehler, Barbara Waidner, Joern Kalinowski, Bernd Kaspers, Stephan Bereswill, & Peter Staeheli

A successful strategy of poxviruses to counteract the host immune response is the expression of soluble cytokine receptors. Using various biological assays we were able to demonstrate the presence of a chicken interferon-gamma (ChIFN-gamma)-neutralising activity in supernatants of fowlpox virus (FPV)-infected cells that could be destroyed by trypsin treatment. The putative ChIFN-gamma binding protein encoded by FPV was partially purified using immobilised recombinant IFN-gamma. Eluted proteins were separated by two-dimensional gel electrophoresis and used for MALDI-TOF MS (matrix-assisted laser desorption/ionisation time of flight mass spectrometer) analysis to determine the molecular masses of peptide fragments generated by tryptic digestion of gel spots containing the putative ChIFN-gamma binding protein. The measured molecular masses of tryptic peptide fragments were then compared with those from virtual tryptic digests of every potential gene product in the FPV genome. This procedure identified the FPV-016 gene as a candidate IFN-gamma receptor. As expected for immune modulatory genes of poxviruses, the 016 gene is located in the terminal region of the FPV genome. In contrast to the known structure of all previously known cellular and poxviral IFN-gamma receptors which contain fibronectin type III domains, the IFN-gamma-binding protein encoded by the FPV 016 gene contains an immunoglobulin domain. These data indicate that fowlpox virus possesses a previously unknown type of soluble IFN-gamma receptor.

Recombinant poxviruses as efficient inducers of primed CD8+ T cells: role of cytokines

Mariano Esteban

Centro Nacional de Biotecnología, Campus de la Universidad Autónoma de Madrid
Cantoblanco, Madrid 28049, Spain

Poxviruses have developed several strategies to subvert the immune system acting at various levels, and in this way establish in the host an effective viral infectious process. In this interplay between the virus and the immune system, cytokines are major players in counteracting virus spreading, and because of that characteristic, this family of viruses encode genes that block cytokine function. Recombinant poxviruses are excellent vaccine candidates against a broad spectrum of pathologies. This is based on the potent immune responses elicited in experimental animals immunized with these viral vectors. There is strong evidence that recombinat poxviruses provide the most efficient immunization strategy for activation and expansion of effector and particularly memory T cells. This has been established with attenuated and replication-deficient poxviruses in prime/booster immunization protocols. To optimize the booster of T cell mediated immune responses by recombinant vaccinia virus (rVV), we have analyzed the role that several cytokines (IL-12, IL-18 and IFN-gamma) exert on rVV replication in tissues and on activation of specific immune responses to antigens from several pathogens (plasmodium, HIV and leishmania). We have identified protocols of cytokine delivery that enhance specific CD8+ T cell immune responses and protection to the pathogen. When IL-12 and IL-18 are delivered by rVVs, different mechanisms involving both the innate and specific arms of the immune system are mediators in the synergistic action of IL-12 and IL-18, leading to VV clearance. However, the delivery by DNA vectors of IL-12, the combination of IL-12+IL-18 or IFN-gamma during DNA prime/rVV boost regimens enhanced the specific immune response to an antigen. This antigen presentation may be stimulated by viral products, like E3L, that enhance translation and cell growth through inhibition of protein kinase PKR. Our findings suggest that immunomodulatory cytokines can be useful in the development of the future vaccines against infectious diseases and tumors.

Session 3: Modulation of cytokine and chemokine responses II
Chair: Grant McFadden

New insights into the modulation of cytokine and chemokine networks by poxviruses and herpesviruses

Antonio Alcami, Margarida Saraiva and Neil A. Bryant

Departments of Medicine and Pathology, University of Cambridge, United Kingdom

Poxviruses and herpesviruses encode unique arrays of proteins that mimic or target key immune modulatory molecules. Characterisation of these viral proteins can teach us about viral pathogenesis, the function of components of the immune system and new strategies of immune modulation.

Ectromelia virus (EV) is a natural mouse pathogen and one of the most virulent poxviruses. The virus causes a generalised disease, known as mousepox, with similarities to human smallpox. EV is being characterised at the molecular level. We have identified a secreted EV protein with similarity to CD30, a member of the tumour necrosis factor receptor superfamily whose role in immune modulation is poorly understood. The viral CD30 homologue (vCD30) does not only act as a decoy receptor blocking CD30-CD30L interactions, but also induces reverse signalling in cells expressing CD30L. vCD30 is a potent inhibitor of Th1-mediated inflammation *in vivo*. This novel immune evasion mechanism uncovers a role for CD30 in anti-viral defence and in type I cytokine-mediated responses.

Chemokines play a key role in recruitment of immune cells to the site of infection. The secretion of chemokine binding proteins (CKBPs) with no sequence similarity to host chemokine receptors is a unique strategy encoded by viruses to modulate chemokine activity. CKBPs have been identified in poxviruses and gammaherpesviruses. A number of chemokines and chemokine receptor homologues have been identified in beta- and gammaherpesviruses, but very limited information is available on the anti-chemokine strategies utilised by alphaherpesviruses. We have identified a family of CKBPs in alphaherpesviruses with no sequence similarity to known viral CKBPs. The CKBP encoded by different alphaherpesviruses shows high amino acid sequence diversity that translates into variable chemokine binding specificity. This family of novel CKBPs provide alternative strategies to block inflammatory responses induced by a variety of chemokines.

Biological properties of virally encoded chemokine ligands, receptors and chemokine binding proteins

Sergio A. Lira

Schering-Plough Research Institute, Kenilworth, NJ, USA

Work in our laboratory focuses on the characterization of the biological function of virally encoded molecules resembling chemokine ligands and receptors. We have used genetic approaches in mice to define the immunomodulatory properties of several chemokine ligands encoded by HHV8 (vMIPI, II and III) as well as the biological properties of the chemokine receptor encoded by HHV8 (ORF74). We will describe several transgenic studies where these molecules have been targeted to particular tissues. In particular, we will discuss studies on the constitutive as well as inducible expression of ORF74 in lymphocytes and in multiple tissues. Expression of ORF74 directed by the CD2 promoter induces an angioproliferative disease resembling Kaposi's sarcoma (KS) (Yang et al., 2000). Recent studies by our lab indicate that the ability of this receptor to induce this KS-like disease is dependent of the modulation of its high constitutive activity by endogenous chemokines (Holst et al., 2001). The implications of these findings to the overall pathogenesis of KS will be discussed. Finally, we will describe our preliminary analysis of transgenic mice expressing M3, a chemokine binding protein encoded by MHV-68. Taken together, these studies provide strong evidence that the virally encoded chemokine-like molecules have powerful biological properties. The use of the genetic models, in particular the inducible models, will facilitate the analysis of these properties during homeostasis and disease.

References:

Yang TY, Chen SC, Leach MW, Manfra D, Homey B, Wiekowski M, Sullivan L, Jenh CH, Narula SK, Chensue SW, **Lira SA** (2000). Transgenic expression of the chemokine receptor encoded by HHV8 induces an angioproliferative disease resembling Kaposi's sarcoma **J Exp Med** 191: 445-454

Holst PJ, Rosenkilde MM, Manfra D, Chen SC, Wiekowski MT, Holst B, Cifire F, Lipp M, Schwartz TW, **Lira SA.** (2001) Tumorigenesis induced by the HHV8-encoded chemokine receptor requires ligand modulation of high constitutive activity. **J Clin Invest** 108:1789-96

The G protein of human respiratory syncytial virus: a receptor binding protein or an immunomodulator

José A. Melero1, B. García-Barreno1, Isidoro Martínez1, Regina García-Beato1, Nuria Rodríguez1, Peter Openshaw2, Geraldine Taylor3 and Gary Bembridge3

1Centro Nacional de Biología Fundamental, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain, 2Imperial College School of Medicine at St. Mary's, London, UK and 3Institute for Animal Health, Compton, UK

The G protein of human respiratory syncytial virus is the receptor-binding (attachment) protein. Two forms of the G protein are expressed in infected cells. The first form is a type II glycoprotein that is made by initiation of translation at the first AUG of the mRNA to produce a membrane-bound protein that is expressed at the surface of infected cells and that it is incorporated in the virus particles. The second form is generated by initiation of translation at a second in-frame AUG that eliminates the first half of the transmembrane region and, after proteolytic processing, is excreted to the culture supernatant.

The membrane–bound form of the G molecule is responsible for binding the virus to the cell receptor at the initial stages of the infectious cycle. Although no specific receptor has been found so far, the G protein can interact with cell surface proteoglycans. This interaction may facilitate the interaction of the G protein with other as yet unidentified high affinity receptors at the cell surface.

The soluble form of the G protein -that encompasses the ectodomain of the membrane-bound form- seems to play an immodulatory role. Thus, soluble G is responsible for priming mice for an eosinophilic response after RSV challenge. This effect seems to be related to the stimulation of certain types of Th cells since elimination of a unique T-cell epitope of the G protein abrogates priming of the eosinophilic response. In addition, the G protein has a CX3C motif that is shared by the chemokine fractalkine and both G and fractalkine can induce leukocyte chemotaxis. Thus, the G glycoprotein may have immunomodulatory activities that seem to be at the basis of the immunopathological response to a RSV infection.

The relevance of these findings for the replicative cycle of RSV and for the immunopathology associated to infections by this virus will be discussed.

Cytomegalovirus-encoded chemokines in pathogenesis

Mocarski, E.S.[1]; Aguirre, S.A.[1]; Schall, T.[2]; Dairhaghi, D.J.[2]; Sparer, T.E.[1]

[1]Department of Microbiology & Immunology, Stanford University
[2]ChemoCentryx, Inc. CA, USA

Human cytomegalovirus (CMV) makes use of a positive-signalling CXC chemokine ligand, vCXC-1 and murine CMV expresses a positive acting CC chemokine homolog, MCK-2, that each attract specific leukocyte populations. Although these represent different classes of chemokines, both are expressed as secreted glycoproteins at late times during productive infection and both induce calcium flux and migration in subpopulations of host leukocytes. Recombinant human CMV vCXC-1 (a 95 aa nonglycosylated protein) signals specifically and solely via the human CXCR2 receptor, one of two IL8 receptors found on human neutrophils. Recombinant vCXC-1 is as potent a chemokine as IL8 on neutrophils but has not been observed to induce migration itself or antagonize migtration to other chemokines in any other population of leukocytes. It may be that vCXC-1 provides a key signal attracting neutrophils to sites of infection where they may acquire virus and disseminate infection to other tissues and organs; however, the species specificity of human CMV prevents a direct test of this hypothesis. We have constructed and characterized a hCXCR2-transgenic mouse with neutrophils that have acquired the ability to respond to this chemokine which should facilitate studies in a host.

We initially characterized of the N-terminal 63 aa chemokine domain of murine CMV MCK-2. A chemically synthesized preparation of this chemokine domain induces calcium flux on murine peritoneal macrophages and macrophage-like cell lines, whereas, a 260 aa full-length recombinant N-Met-MCK-2 (representative of the natural chemokine) does not. Recombinant N-Met-MCK-2, a nonglycosylated protein with an nonnatural N-terminal methionine, was evaluated for ability to induce a calcium flux on murine leukocyte populations. Although N-Met-MCK-2 failed to signal to any major population of leukocyte present at the footpad inoculation site, in peripheral blood or in spleen, the protein may signal to a minor population of murine leukocytes whose phenotype is under evaluation.

When the behavior of *mck+* and *mck-* viruses is compared in infected mice, the first obvious difference is that expression of MCK-2 confers a higher level of swelling at a footpad inoculation site attributable to alterations of both edema and cellularity. Twice the number of inflammatory cells are recovered from *mck+* compared to *mck-* virus-infected footpads at day 2 or 3 post inoculation even though virus replication levels are equivalent. Based on FACS analysis of cell surface markers (MHC class II, Mac-1, Mac-3, CD40, CD11c, pan-NK, CD4 and CD8) at day 3, the proportion of leukocyte types is similar whether or not MCK-2 is expressed. The second, more striking observation is that the increased innate response resulting from expression does not increase the rate of viral clearance at the inoculation site or anywhere else in the host that has been examined. Third, expression of MCK-2 contributes to a 20-fold increased level of peak viremia and a 1000-fold increased peak levels of virus in salivary glands without affectin the levels of virus in lymph nodes, spleen, liver or lungs and without modulating the contribution of either NK or adaptive (T or B) cell-mediated immunity to the control of infection.

Inoculation with recombinant N-Met MCK-2 induces an inflammatory mononuclear cell response following injection of footpads either alone or together with virus. Administration of this recombinant protein with virus increases the level of swelling induced by *mck-* virus to levels of wild type virus. Co-administration of the recombinant protein induces increased swelling of *mck+* viruses as well, but this treatment does not result in increased levels of viremia or dissemination to salivary glands. The cell population that is recruited by N-Met-MCK-2 in the host is under evaluation and is enriched for CD40 but not for other dendritic cell, macrophage or B cell markers. These studies highlight a role for virus-encoded chemokines in attracting host leukocytes that increase the efficiency of dissemination.

Localization of the human cytomegalovirus UL33 and US27 proteins in endocytic compartments and viral membranes

Alberto Fraile-Ramos

The human cytomegalovirus (HCMV) genome encodes four 7 transmembrane domain chemokine receptor-like proteins (1). Although important in viral pathogenesis (2), little is known about the properties of these proteins. We previously reported that US28 is located in endocytic vesicles and undergoes constitutive endocytosis and recycling (3). Here we studied the cellular distributions of two other HCMV chemokine receptor-like proteins, UL33 and US27, in transfected and HCMV infected cells. Immunofluorescence staining indicated that UL33 and US27 are located at the cell surface but the majority of both proteins is seen to be intracellular. The intracellular localization is perinuclear and showed overlap with markers for endocytic organelles. By immunogold electron microscopy (EM), UL33 was seen to localize to multivesicular endosomes.

EM analysis of HCMV infected cells showed that most virus particles wrapped individually into short membrane cisternae. In addition, some virus particles were also seen within and budding into multivesicular bodies (MVBs). EM immunolocalization of viral UL33 and US27 showed gold particles in the membranes into which virions were wrapping, in small tubules and vesicles and in MVBs. Labeling of the HCMV glycoproteins gB and gH indicated that these proteins were also present in the same membrane structures. Together these observations suggest that the localization of UL33, US27 and US28 to endosomes may allow these proteins to be incorporated into the viral membrane during the final stages of HCMV assembly.

References:

1.- Chee et al. (1990) Nature. 344, 774-777.
2.- Rosenkilde et al. (2001) Oncogene. 20, 1582-1593.
3.- Fraile-Ramos et al. (2001) Mol Biol Cell. 12, 1737-1749.

The role of antibody responses and target cell membrane organization in HIV-1 infection

Carlos Martínez-A., Gustavo del Real, José L. Torán and Santos Mañes

Dept of Immunology & Oncology, Centro Nacional de Biotecnología/CSIC, UAM Campus de Cantoblanco, E-28049 Madrid Spain

HIV-1 induces $CD4^+$ T lymphocyte depletion and a subsequent acquired immune deficiency syndrome (AIDS) in the host. Virus entry into host cells is mediated by the viral envelope glycoprotein 120 (gp120) and the transmembrane glycoprotein 41 (gp41) together with two distinct cell surface receptor molecules, CD4 and a specific chemokine receptor. CCR5 is used preferentially by most primary isolates, and CXCR4 by laboratory strains and some primary isolates. To better understand virus-target interaction, we have analyzed the antibody response of long-term non-progressor HIV-1-infected individuals, as well as the cell membrane organization required for maintenance of the CD4/chemokine receptor complex. The two cell surface receptor molecules, CD4 and the chemokine receptor required for HIV-1 infection, are located in highly sophisticated structures composed of distinct lipid domains, termed rafts, that functionally organize the proteins embedded in the bilayer.

The humoral response to gp120 was analyzed using phage display libraries constructed from donors; this shows an IgM response comprised of low affinity polyreactive antibodies that mature to a more competent secondary IgG response. High affinity IgG Fab fragments, in contrast, are Vh-restricted and specific for gp120; they were able to neutralize several reference viral strains, including X4 and R5, both *in vitro* and *in vivo*. One of the IgG Fabs tested (S8) showed *in vivo* neutralizing activity against M-tropic (BaL) HIV-1 virus, using human PBL-reconstituted SCID mice as a viral infection model. Peptide mimotopes able to compete for Fab-gp120 binding were selected from random peptide phage display libraries. Mimopeptide information and molecular modeling of gp120 and antibody structure were used to define the S8 Fab epitope and S8 Fab. We identified a hypothetical interaction between Arg95 in the Fab S8 HCDR3 loop and Glu381 in gp120 that may be relevant for the changes in the gp120 inner-outer interdomain relationships. The model suggests that the Fab epitope is conformational and involves key gp120 residues implicated in the chemokine coreceptor binding site.

In an additional study, we analyzed the implications, in infection by HIV-1, of the spatial segregation of HIV-1 receptors into different lipid environments. Whereas the role of rafts in CD4-mediated signaling is well established, the importance of the chemokine receptors and CD4 association with rafts for HIV-1 entry remains a subject of debate. We found that the HIV-1 coreceptors CCR5 and CXCR4 partition in rafts after ligand- or gp120-induced clustering. Raft disruption by membrane cholesterol sequestration blocks HIV-1 entry; this inhibition correlates with the absence of CD4-gp120-CXCR4 complexes on the surface of cholesterol-depleted cells. This suggests that large CD4-gp120-coreceptor complexes are the consequence of raft clustering initially induced by HIV-1 binding to CD4. Even assuming that CD4 and CCR5 may be constitutively associated at the cell surface, HIV-host membrane fusion is a cooperative process that requires multiple CD4-gp120-CCR5 complexes that can be formed by lateral raft rearrangement. Viral entry would thus be prevented by CD4 and chemokine receptor partitioning outside of raft domains.

Session 4: Modulation of cytotoxic T cell and natural killer cell responses I
Chair: Hidde L. Ploegh

Effects of hCMV US2 and US11 proteins on the induction of virus specific CD8+ T lymphocytes *in vivo*

Jonathan W. Yewdell

Cellular Biology Section Laboratory of Viral Diseases
National Institute of Allergy and Infectious Diseases

The extent to which naïve CD8+ T cells (T_{CD8+}) are primed by APCs presenting endogenous antigens (direct priming) or antigens acquired from other infected cells (cross-priming) is a critical topic in basic and applied immunology. To examine this question we used vaccinia viruses that express human cytomegalovirus proteins (US2 or US11) that induce the destruction of newly synthesized MHC class I molecules. Expression of US2 or US11 was associated with a 25-70% decrease in numbers of responding primary or secondary VV-specific T_{CD8+}. Using HPLC-isolated peptides from VV-infected cells, we found that US2 and US11 selectively inhibit T_{CD8+} responses to a subset of immunogenic VV determinants. The same subset of peptides fail to induce T_{CD8+} responses when VV-infected histoincompatible cells are used to cross-prime for VV-specific T_{CD8+}. These findings indicate that first, US2 and US11 function in vivo to interfere with the activation of virus-specific T_{CD8+}, second, cross-priming and direct priming contribute to the generation of VV-specific T_{CD8+}, and third, the sets of immunogenic determinants generated by cross-priming and direct priming are not completely overlapping.

Multiple alternative protease systems can process viral antigens for presentation to $CD8^+$ T lymphocytes

Margarita Del Val, D. López, B. C. Gil-Torregrosa, A. R. Castaño, Y. Samino
M. Rodríguez-Castro, P. De León, S. Guil, and M. Montoya

Centro Nacional de Biología Fundamental. Instituto de Salud Carlos III
E-28220 Madrid. Spain

Recognition of virus-infected cells by $CD8^+$ cytotoxic T lymphocytes (CTL) represents a critical mechanism for virus clearance from the infected host. Viral proteins synthesized within an infected cell are proteolytically processed by proteases. Peptide products are then bound by nascent MHC class I molecules, which transport them to the cell surface for display and recognition by CTL.

The proteasome, whose major role is the degradation of unneeded proteins, is the most abundant and multicatalytic of cellular proteases. As a consequence, it has been considered as the major source of viral peptide epitopes for CTL, even though it has not been evolutionary selected for this task. Our hypothesis is that any other cellular protease, whose primary role is also not viral antigen processing, may also contribute to generating MHC class I ligands and thus expand the repertoire of possible peptides displayed by infected cells to signal them for recognition and elimination by CD8+ T lymphocytes.

We present evidence of several alternative protease systems involved in viral antigen processing for presentation to CTL. One of them is mediated by ***furin***, a trans-Golgi-resident serin protease that cleaves after polybasic residues. The pathway is independent of proteasomes and TAP, and takes place entirely in the secretory pathway. A second viral antigen processing pathway, involving ***metalloproteases***, is sequential to the activity of proteasomes, takes place in the cytosol, and requires functional TAP.

Targeting the function of mature dendritic cells by human cytomegalovirus: a multilayered viral defense strategy

Martin J. Raftery,[1] Henning Walczak, H.,[2] Günther Schönrich[1]

[1]Institute of Virology, Charité Medical School, Humboldt University Berlin
[2]Tumor Immunology Program, Department of Apoptosis Regulation,
German Cancer Research Center (DKFZ), Heidelberg

Dendritic cells (DC) play a pivotal role in initiating the antiviral immune response. Therefore, interactions of viruses with DC are crucial for the outcome of viral infections and can have different consequences ranging from immunostimulation to immunosuppression. For example we observed that hantaviruses can infect DC thereby inducing DC maturation. In contrast other DC-tropic viruses significantly alter DC phenotype and function. We have found that DC can be infected by human cytomegalovirus (HCMV) which can then suppress the immune system. HCMV-infected DC show slightly enhanced expression of co-stimulatory molecules (CD40, CD80, CD86). In contrast, MHC class I and II molecules are partially down-regulated, which leads to a reduced antigen presenting capacity. In addition, the apoptosis-inducing ligands CD95L (FasL) and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) are up-regulated thereby enabling HCMV-infected DC to delete activated T lymphocytes. In comparison to down-regulating of the antigen presentation these death-inducing and silencing mechanisms represent a more aggressive strategy by which a virus can disable the host immune defense. Moreover, T cells that were not eliminated by induction of apoptosis were functional silenced. Thus, infection of DC allows HCMV to blunt the antiviral T cell response by a multi-layered defense strategy which may significantly contribute to HCMV-associated immunosuppression *in vivo*.

The role of the Natural Killer Complex (NKC) in controlling large DNA viruses

Anthony A. Scalzo

Dept of Microbiology, University of Western Australia, Nedlands, WA, 6009, Australia

While many herpesviruses and poxviruses have evolved a range of pathogenic strategies to maximise their replicative success in their host organism, including sophisticated immune evasion mechanisms, the host is also well equipped to cope with infection by mounting a range of innate and adaptive immune responses. Natural killer (NK) cells represent an important frontline defense strategy against a range of pathogens including viruses, bacteria and parasites.

Infection of mice by mouse cytomegalovirus (MCMV) represents one of the best-characterised models for demonstrating the importance of NK cells during virus infections. In this model, the *Cmv1* host-resistance locus regulates NK cell control of MCMV replication in the spleen and bone marrow (1, 2). Data indicate that *Cmv1* predominantly regulates the cytolytic functions of NK cell activity. *Cmv1* is located on mouse chromosome 6 in the NK gene complex (NKC). This region encodes cell many surface molecules predominantly expressed on NK cells and which function as either activation or inhibitory receptors. Linkage studies using resistant (C57BL/6J) and susceptible (BALB/c, DBA/2J) inbred strains of mice showed that *Cmv1* is most closely linked to the *Ly-49* family of activation and inhibitory NK cell receptors (3, 4). Recent studies focussed on the molecular identification of the gene that encodes *Cmv1*. Using the BXD8 recombinant inbred mouse strain we found that this mouse has a deletion in the B6-derived Ly49H gene and is thus susceptible to infection. Furthermore, monoclonal antibodies (mAbs) to Ly49H can specifically abrogate the NK cell-dependent restriction of virus replication in B6 mice (5, 6). Together, this genetic and biological data indicate that the Ly49H gene mediates the major *Cmv1* effect.

Herpes simplex virus 1 (HSV-1) pathogenesis has also been widely assessed using mouse models with infection being controlled by at least 4 host resistance loci (7). Inbred strains differ in their susceptibility to infection with B6 mice being resistant and BALB/c susceptible. Using the BALB.B6-*Cmv1*^{r} congenic strain we have shown that resistance of B6 mice is in part contributed to by a resistance locus (*Rhs1*) residing in the NKC region. In addition, treatment of resistant BALB.B6-*Cmv1*^{r} mice with anti-NK1.1 mAb increases virus titres in dorsal root ganglia suggesting NK or NKT cells may be involved in mediating protection (8). Use of a subpanel of intra-NKC recombinant strains derived from the BALB.B6-*Cmv1*^{r} congenic has shown that *Rhs1* is distinct from, and maps proximally to, *Cmv1*.

Like MCMV and HSV-1, ectromelia virus (EV) infection of mice leads to genetically determined differences in resistance to infection in C57BL/6J (resistant) and BALB/c and DBA/2J (susceptible) strains. One of the 4 loci conferring genetically determined resistance to EV is *Rmp1*, which Delano and Brownstein (9) have shown to be linked to the NKC region. Using the BALB.B6-*Cmv1*^{r} congenic strain we have confirmed *Rmp1* is NKC linked and that *Rmp1*-mediated resistance in this strain can be abrogated by anti-NK1.1 treatment.

Given the linkage to *Cmv1* we explored whether anti-Ly49H mAb treatment modulated EV resistance. We found no effect, suggesting the *Rmp1* locus is distinct from *Cmv1*.

Combined these data indicate that while the NKC region exerts genetically determined effects on host innate immune control of different herpesvirus and poxvirus members, this is via different loci in this region and hence via distinct mechanisms.

References:

1. Scalzo, A.A., N.A. Fitzgerald, A. Simmons, et al. 1990. ***J. Exp. Med.*** *171:1469.*
2. Scalzo, A.A., N.A. Fitzgerald, C.R. Wallace, et al. 1992. ***J. Immunol.*** *149:581.*
3. Forbes, C.A., M.G. Brown, R. Cho, et al. 1997. ***Genomics*** *41:406.*
4. Depatie, C., E. Muise, P. Lepage, et al. 1997. ***Genomics*** *39:154.*
5. Brown, M.G., A.O. Dokun, J.W. Heusel, et al. 2001. ***Science*** *292:934.*
6. Lee, S.H., S. Girard, D. Macina, et al. 2001. ***Nature Genetics*** *28:42.*
7. Simmons, A., and A.B. La Vista. 1989. ***Virus Research*** *13:263.*
8. Pereira, R.A., A. Scalzo, and A. Simmons. 2001. ***J. Immunol.*** *166:5869.*
9. Delano, M.L., and D.G. Brownstein. 1995. ***J. Virol.*** *69:5875.*

Co-operative functions of the multiple immune evasion genes of murine cytomegalovirus (MCMV)

Ann B. Hill, Daniel G. Kavanagh, Marielle Gold, Diane LoPicollo, Markus Wagner and Ulrich Koszinowski

A striking feature of the immune evasion strategies of cytomegloviruses is the multitude of genes they encode with seemingly redundant functions. MCMV encodes at least three genes known to interfere with the MHC class pathway of antigen presentation: m152 causes class I to be retained in the ER cis-Golgi intermediate compartment, m6 redirects class I to the lysosome for destruction, and m4gp34 forms two distinct types of complexes with class I molecules- in a pre-Golgi compartment and at the cell surface. We have analyzed the contribution of these genes to interference with antigen presentation using biochemical and functional analyses. We have found that one function of the multiple genes is to cope with the diversity of class I molecules present in an outbred population. M152 is highly effective against the class I molecule Db but only partially effective against Kb. In consequence, complete abrogation of target cell recognition by CTL for Kb-restricted CTL requires the presence of all three immune evasion genes, whereas Db is completely blocked by m152 alone. Another reason for multiple genes is revealed in different cell types: in fibroblasts, m152 is dominant, but in macrophages, the contributions of m4 and perhaps m6 are at least as important.

Protection against poxvirus infections: The roles of cytokines, cytotoxic T lymphocytes and antibody

Gunasegaran Karupiah

Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University

I have determined the in vivo effector function of CD8 T lymphocytes that is critical for clearance of certain poxviruses. There is still some question of whether it is their cytolytic potential or their ability to produce cytokines which is important. Indeed, it has been proposed that recovery from infection with cytopathic viruses requires cytokines like IFN-γ or antibody but not CTL whereas non-cytopathic viruses require CTL but not antibody or cytokines. My investigations using ectromelia virus (EV), a cytopathic virus, indicate that recovery from a primary infection with this virus clearly requires CTL (perforin-mediated cytolysis), IFNs (IFN-α, -β and -γ), and most intriguingly, antibody. In addressing the role of these immune parameters in recovery from a secondary EV infection, I have found that only antibody is critical for protective immunity. This finding has important implications for vaccination strategies. The requirement for cell-mediated immunity, cytokines and antibody for recovery from a primary viral infection appears unrelated to whether the virus is cytopathic or non-cytopathic.

To further address the relative importance of perforin-dependent cytolytic activity and IFN-γ in virus clearance, an adoptive transfer model using immune cells generated in mice lacking either perforin or IFN-γ function was utilized. Mutant mice were immunized with avirulent EV or vaccinia virus (VV). Unlike cells from wild-type mice, immune effe1tors transferred from perforin-deficient mice were unable to control infection with EV. However, IFN-γ-deficient effectors cleared infection as efficiently as wild-type cells. In contrast, control of VV infection was critically dependent on IFN-γ production by transferred cells, but occurred largely independently of perforin production. The antiviral activity of the transferred effector cells was mediated by the CD8+ T cell population. Taken together, our results illustrate the different requirements for antibody, IFN-γ and perforin function in control of infection with the poxviruses VV and EV.

Session 5: Modulation of cytotoxic T cell and natural killer cell reponses II
Chair: Margarita Del Val

Genetic approach to herpesviral immune evasion and pathogenesis

Dr. Ulrich H. Koszinowski

Max von Pettenkofer-Institut
Pettenkoferstraße 9a
80336 München

Cytomegalovirus (CMV) defines the β subgroup of herpesviruses. The 240 kb genome of CMV comprises about 200 open reading frames. Only a minority of these genes have been analyzed. The majority of the genes is not necessary for producing infectious herpesvirus. The genes probably control virus-cell interactions and the interaction with the immune system of the host. Genes which show homology with mammalian genes are subjects of at depth analysis. Other genes which may affect the function of host cell gene products can only be found after description of a specific phenotype seen in infected cells. In the past we have focussed on methods which can be used for the identification of genes which affect the immune response of the host or the biology of the infected cell. As many of the evasion genes have only been studied so far on the level of the isolated gene, the activity in the genomic context has not been fully explored yet. Here we describe the cooperation / competitivity of some immune evasive genes in the genomic context and contribute information on other genes which apparently affect the cell type specificity of herpesviruses.

The MCMV m152/gp40 glycoprotein downregulates expression of a cellular ligand for the NKG2D receptor and inhibits NK cell-mediated virus control *in vivo*

Astrid Krmpotic*, Dirk H. Bush**, Ivan Bubic*, Hartmut Hengel***, Milena Hasan*, Anthony A. Scalzo****, Ulrich H. Koszinowski***** and Stipan Jonjic*

*Faculty of Medicine, Rijeka, Croatia, **Institute for Microbiology, Immunology and Hygiene, Technical University Munich, Germany, ***Robert Koch-Institute, Berlin, Germany, ****Department of Microbiology, University of WA, Nedlands, Australia and *****Max von Pettenkofer Institute, Munich, Germany

The sensitivity of some mouse strains (e.g. BALB/c) to murine cytomegalovirus (MCMV) is related to their inability to generate an efficacious NK response during the early post infection period. In this study we tested whether the MCMV-sensitive mouse strains are constitutively unable to mount an NK response to this virus or if their sensitivity is due to viral inhibition of NK cell function. We present the evidence that m152/gp40, originally described as a gene that compromises CD8+ T cell function by retaining the MHC class-I complexes in the ERGIC/cis-Golgi compartment, also downmodulates the surface expression of ligands for the NKG2D receptor, resulting in the inhibition of NK cell-mediated control of virus growth *in vivo*. Furthermore, virus growth is restricted by NK cells in otherwise-susceptible mice infected with a virus harboring a deletion of the m152 gene. Thus, a single herpesviral protein can function to downmodulate both adaptive and innate immune responses.

Inter-relationships between HIV and the immune response

Andrew J. McMichael

MRC Human Immunology Unit
John Radcliffe Hospital
Oxford OX3 9UT UK

HIV has devastating effects on the immune system of infected people yet provokes and apparently strong T cell response to the virus. In the acute phase of virus infection there is a strong CD8+ T cell response, and later a neutralising antibody response. Although the CD8+ T cell response is numerically strong it does differ in phenotype and function from other those in other virus infections (1). Lytic activity is impaired and given that the nef protein of HIV down-regulates HLA A and B expression, this could impair control of the infection. In the acute phase and chronic infection the CD8+ T cells select virus escape mutants and this plays a major part in progression of the infection (reviewed in(2)). At the same time the CD4+ T cell response is undermined by the virus infecting activated CD4+ T cells, including those specific for HIV. HIV also damages dendritic cells. Patients who have slow progression of disease have stronger CD4+ and/or CD8+ T cell responses (3). As CD4+ T cells decline drastically in number, the helper function necessary for maintenance of CD8 T cell responses declines and ultimately the whole immune response collapses.

HIV undermines immune responses by infecting and damaging vital immunocytes. In addition virus proteins contribute. particularly nef. Nef activates T cells, down-regulates CD4 and HLA class I (A and B) expression and up-regulates Fas ligand. All of these effects contribute to the failure of the immune response to control this infection (reviewed in (4)).

The virus variability that is partly a consequence of poor immune control presents major problems to vaccine design. It has not been possible to design antigens that induce strong neutralising antibody responses – the envelope protein gp120 has structural features that facilitate immune escape. Internal virus proteins differ by 10-20% between major virus subtypes, sufficient to greatly impair cross-clade recognition by CD8+ T cells. Virus variability is likely to be the major hurdle in the generation of effective vaccines for prophylaxis and therapy.

References:

1. Appay, V., D.F. Nixon, S.M. Donahoe, G.M. Gillespie, T. Dong, A. King, G.S. Ogg, H.M. Spiegel, C. Conlon, C.A. Spina, D.V. Havlir, D.D. Richman, A. Waters, P. Easterbrook, A.J. McMichael, and S.L. Rowland-Jones. 2000. HIV-specific CD8(+) T cells produce antiviral cytokines but are impaired in cytolytic function. *J Exp Med* 192:63-75.

2. McMichael, A. 1998. T cell responses and viral escape. *Cell* 93:673-676.

3. Rosenberg, E.S., M. Altfeld, S.H. Poon, M.N. Phillips, B.M. Wilkes, R.L. Eldridge, G.K. Robbins, R.T. D'Aquila, P.J. Goulder, and B.D. Walker. 2000. Immune control of HIV-1 after early treatment of acute infection. *Nature* 407:523-526.

4. Xu, X.N., G.R. Screaton, and A.J. McMichael. 2001. Virus infections. Escape, resistance, and counterattack. *Immunity* 15:867-870.

Inhibition of the MHC class II antigen presentation pathway by HCMV glycoproteins US2 and US3

David C. Johnson, Nag Hegde, M. Chevalier, C. Dunn

Department of Molecular Microbiology & Immunology, Oregon Health Sciences University, Portland, OR 97201

Human cytomegalovirus (HCMV) expresses at least 8 small glycoproteins from the US2-US11 region that are all retained in the ER or reach the Golgi apparatus, but not the cell surface. Four of these glycoproteins, US2, US3, US6 and US11 have previously been shown to inhibit the MHC class II antigen presentation pathway. We demonstrated that US2, not only causes degradation of class I proteins, but also targets class II DR-alpha and DM-alpha for proteasome mediated degradation. DR-ß or DM-ß were not affected, and this effect has been observed in several cell lines and when DR-alpha or DR-ß were transfected into cells alone. US2 shows a small preference for class I HC over class II DR-alpha, amounting to 1.5-2 fold when US2 is limiting in cells that express both class I and II. There is evidence that cellular factors other than Sec-61 and class I or II proteins are involved in this degradation pathway and are limiting. We extended these observations by expressing 7 of the 8 US2-US11 glycoproteins in U373/CIITA cells, observing that both US2 and US3 inhibited recognition by CD4+ T cells. US3 inhibits the class II pathway by a novel mechanism, binding to class II complexes early after their synthesis and reducing assembly with invariant chain, Ii. As a result class II complexes produced in US3 expressing cells were mislocalized in cells, inefficiently delivered to lysosomal/MIIC compartments and loading of antigenic peptides was reduced bg 3-9 fold. Therefore, we have identified two HCMV glycoproteins that bind to class II proteins, US2 causes degradation and US3 mislocalizes class II. Domain swaps between US2 and US3, as well as truncated versions of US2, have been constructed in order to map regions involved in binding and in degradation.

HHV-6 modulates the surface expression of key molecules of the immune system

Patricia Fuentes*, Rafael Bragado* and Begoña Galocha[¶]

* Fundación Jiménez Díaz, [¶] Hospital Clínico San Carlos. Madrid. Spain

Human Herpesvirus 6 (HHV-6) infects cells of the immune system and thus may modulate their function. The present study was undertaken to investigate the potential immunomodulatory effects of this virus. Uninfected HSB-2 cells were cultured with the same cell line infected with HHV-6. Viral replication was detected by immunofluorescence and RT-PCR analysis. We studied the effect of HHV-6 infection on the cell surface expression of various activation markers (CD69, CD25 and MHC class II). Marked down-regulation of CD69, CD25 and class II expression was detected in HHV-6 infected cells as evidenced by flow cytometry analysis. By investigating the mechanisms involved in the down-regulation of CD69 and CD25, RT-PCR analysis showed that CD69 and CD25 mRNAs were reduced in infected cells.

We also studied how HHV-6 interferes with MHC class I expression. Cytotoxic T lymphocytes eliminate virally infected cells. They recognize virus derived peptides in association with MHC class I antigens. Viruses avoid detection by the immune system by modulating MHC class I expression. MHC class I expression was down-regulated in HHV-6 infected cells as evidenced by flow cytometry. RT-PCR analysis show that MHC class I mRNA is not reduced in infected cells. Pulse-chase and immunoprecipitation analysis of metabolically labeled infected cells demonstrate that HHV-6 interferes with MHC class I presentation pathway after MHC molecules leave the endoplasmic reticulum. Kinetics studies of the MHC class I export to the cell surface show that this is delayed in cells infected with HHV-6. Pulse-chase and immunoprecipitation analysis of metabolically labeled infected cells also demonstrate that MHC class I molecules are directed to lysosomal compartments for destruction in HHV-6 infected cells.

The results demonstrate that infection with HHV-6 results in immune supression, characterized by a profound down-regulation of CD69, CD25 and MHC class I and class II molecules.

Viral immune evasion by HCMV

Hidde L. Ploegh

The mechanisms that underlie immune evasion, as practiced by HCMV, revolve around a set of genes, the US cluster, that includes US2 and US11. The US2 and US11 proteins target MHC ClassI molecules for rapid degradation. Recent progress in the study of the underlying mechanisms will be presented.

POSTERS

New targets for manipulation of cell apoptosis regulation by viruses

P. Fernandez-Zapatero 1, B. Hernáez 1, L. Dixon 2, J.M. Escribano and C. Alonso 1

1 Dpto. Biotecnología, INIA, Madrid, Spain; 2 Institute for Animal Health, Pirbright, U.K.

Viruses have evolved strategies to modify host cell functions and the immune response. Apoptosis regulation in the host cell ensures viral replication and may constitute an effective viral escape mechanism. Interfering with the cell death program, viruses can alter cell viability of immune defense elements.

African swine fever virus (ASFV), is a large DNA virus encoding apoptosis regulator genes which exert their action either counteracting cellular bcl-2 family of genes such as the viral bcl-2 homolog, A179L does, or sequestering anchor proteins of caspase precursors or by blocking apoptogenic molecules exit from mitochondria.

The use of cytoplasmic transport of the cell during early steps of infection, enables the virus to reach the replication site. Once delivered into the cytosol, virions have to be transported to sites of replication, and some viruses use microtubule motors to move from the cell periphery along the cytosol. We have characterized targeting signals that incoming virions expose on their surface for association with molecular motor complexes. ASFV hijacks the microtubule motor complex machinery during virus infection through direct binding of virus protein p54 to a light chain of cytoplasmic dynein. A 13 -amino-acid domain was sufficient for dynein binding, a 3 amino-acid motif being critical for this binding. Interaction occurs both in vivo and in vitro and the two proteins co-localize in a paranuclear location. A number of both RNA and DNA viruses currently studied share this targeting signal thus dynein -binding could constitute a widespread mechanism for virus trafficking that might be considered in the design of new antiviral drugs. Identification of cellular targets of viral genes modifiers of apoptosis, also provide novel targets and strategies for development of drugs controlling cell proliferation.

The epidemic keratokonjunctivitis causing adenovirus 19a secretes a novel leukocyte-binding E3 protein

Mark Windheim1, Christine Falk2, Elisabeth Kremmer2 and Hans-Gerhard Burgert1

1Max-von-Pettenkofer Institute, Gene Center, Ludwig-Maximilians-University, Feodor-Lynen-Str. 25, 81377 Munich, Germany, 2GSF, Institute of Immunology, Munich, Germany
email: burgert@lmb.uni-muenchen.de

The early transcription unit 3 (E3) of human adenoviruses (Ads) encodes proteins with immunomodulatory function. We previously discovered a novel ORF of 49K within the E3 region of Ad19a, an Ad that causes epidemic keratoconjunctivitis. We recently showed that 49K is present in all subgenus D Ads examined (1). The sequence predicts a highly glycosylated type I transmembrane protein. Using antibodies directed to the C-terminus of 49K, a protein of 80-100 kDa containing 12 and 13 N-linked glycans as well as O-linked glycans was precipitated. Synthesis of 49K begins in the early phase of infection and continues throughout the infection cycle. In the early phase, 49K is primarily localized in the Golgi/trans Golgi network but also in early endosomes and at the plasma membrane. In the late phase 49K is also found in late endosomes/lysosomes (2).

Interestingly, in pulse-chase analyses we also detect 49K fragments (Mr: ~12 kDa) derived from the C-terminus. To investigate the fate of the N-terminal portion of 49K, we raised new antibodies against this domain. With these antibodies, we demonstrate that the large N-terminal part of 49K is not degraded but is secreted. Secretion of the 49K ectodomain is a novel phenomenon for Ad E3 proteins. In contrast to all known E3 proteins, which act on the infected cell, 49K is likely to affect surrounding cells. In support of this notion, 49K was found to bind to lymphocytes. We hypothesize, that secreted 49K functions as a regulatory factor for infiltrating cells of the immune system.

References:

(1) Blusch et al. (2002) Virology, manuscript in press.
(2) Windheim, M. and Burgert, H.-G. (2002) J. Virol., manuscript in press.

Identification and expression of two human cytomegalovirus transcription units coding for distinct Fcg-receptor homologs

Ramazan Atalay1, Albert Zimmermann1, Markus Wagner2, Eva Borst2, Christine Benz2, Martin Messerle2, and Hartmut Hengel1

1Robert Koch-Institut, Fachgebiet Virale Infektionen, 13353 Berlin, Germany
2Max von Pettenkofer-Institut, Lehrstuhl Virologie, 81377 München, Germany

Cellular receptors for the Fc domain of IgG (cFcgR) comprise a family of surface receptors on immune cells connecting humoral and cellular immune responses. Several herpesviruses induce FcgR activities in infected cells. Here we identify two distinct human cytomegalovirus (HCMV) AD169 encoded vFcgR glycoproteins, gp34 and gp68. A panel of HCMV strains exhibited slight molecular microheterogeneity between Fcg-binding proteins, suggesting their viral origin. To locate the responsible genes we constructed a large set of targeted HCMV deletion mutants. Mutant analysis located the UL115-UL119 transcription unit to express gp68, and the genomic region of TRL10 through TRL14 to express gp34, respectively. Cloning, sequencing, expression and functional analysis of UL115-UL119 derived cDNAs proved a spliced UL119-UL118 mRNA to encode gp68, a surface resident type I transmembrane glycoprotein. Likewise, expression of IRL11 resulted in a glycoprotein with Fcg binding properties and cell surface exposition. Alignment of gp68 and gp34 sequences with cFcgRs predicts the presence of a conserved single IgSF V-like domain positioned in the N terminal part of each vFcgR extracellular chain. Sequence relatedness of gp68 and gp34 with particular domains of FcgR I and FcgRs II/III, respectively, suggests different ancestry and function of vFcgRs, and highlights the impressive diversification and redundancy of FcgR structures.

Characterization of a chemokine receptor from Yaba-like disease virus

Najarro, P., Lee, H. J. & Smith, G.L.

Wright Fleming Institute, Imperial College of Science, Technology and Medicine, St. Mary's Campus, Norfolk Place, London, W2 1PG

The genome of Yaba-like disease virus (YLDV) was sequenced recently (Lee et al., 2001). Computational analysis revealed two predicted proteins (7L and 145R) with a high degree of amino acid similarity to the seven transmembrane (7TM) G-protein coupled receptor CC chemokine receptor 8 (CCR8). Several viruses encode 7TM proteins related to chemokine receptors but in most cases the functional significance of these proteins has not been determined and their ligands are unknown.

We tested the ability of YLDV-infected cells to bind 125I-CCL1and found that: (i) YLDV infected cells bound to 125I-CCL1in a dose dependent manner (IC50 ~ 7 nM); (ii) the binding activity could be detected from 2 h post-infection even; (iii) the chemokine-binding pattern of YLDV-infected cells is promiscuous since addition of several CC chemokines [vMIPI, vMIPII, CCL5 (RANTES), CCL7 (MCP-3), CCL17 (hTARC) and CCL3 (MIP-1a)] inhibited binding of radiolabelled CCL1 to the YLDV-infected cells.

To determine if CCL1 was binding to 7L, 145R or both proteins, and to characterize their mode of action, each protein was expressed independently in a vaccinia virus (WRDB8R attenuated strain) either as the wild type protein or variants in which the YLDV proteins were tagged at the N or C terminus with an HA epitope recognised by a MAb. Both 7L and 145R are associated with the plasma membrane. Preliminary binding studies with 125I-CCL1 indicated that 7L binds to the CCR8 ligand CCL1. Viral 7TM receptor 7L also exhibited affinity to vMIPII, CCL5 (RANTES) and CCL3 (MIP-1a).

Cytoplasmic C-termini tails of 7L and 145R are shorter than their host counterpart, which might result in a dominant negative effect on CCR8 signal transduction events during YLDV infection. Studies on downstream signalling and overall function of CCR8 in the context of 7L and 145R expression will be addressed using mammalian cell lines expressing these receptors. In summary, we demonstrate the YLDV protein 7L can bind several CC chemokines. The role of this protein in cell signalling, virus virulence and the immune response to infection are being investigated.

Reference:

Lee, H.-J., Essani, K. & Smith, G.L. (2001). The genome sequence of Yaba-like disease virus, a yatapoxvirus. Virology, 281, 170-192.

Expression of the UL16 glycoprotein of human cytomegalovirus is associated with protection of the virus-infected cell from attack by natural killer cells

Mar Valés Gómez, Helena Browne, Tony Minson and Hugh Reyburn

Department of Pathology. University of Cambridge, Tennis Court Road
Cambridge CB2 1QP, UK

Human Cytomegalovirus (HCMV) has evolved/acquired an extensive repertoire of proteins that are dispensable for virus replication *in vitro*, but which maybe important for virus survival *in vivo*. Many of these proteins seem to have a function in viral immune evasion strategies directed against humoral immunity (a virally encoded Fc receptor [1]), T cell mediated immune surveillance (a set of proteins which interfere with various steps in antigen processing and presentation by MHC Class I and Class II molecules) and Natural Killer (NK) cells [2]. HCMV strategies to evade NK cell immune surveillance include encoding gene products able to engage NK inhibitory receptors (e.g. UL18 [3] and UL40 [4]) as well as viral molecules able to block the interaction of target cell ligands, induced by CMV infection, with NK activating receptors (UL16 [5]). The significance of these potential immune evasion strategies is not clear since in functional experiments neither UL18 nor UL40 protected virus-infected cells from NK cell attack ([6, 7]), while the experiments demonstrating that UL16 could block the binding of the NK cell activating receptor NKG2D to target cell expressed ligands were done with recombinant soluble UL16 protein ([8]). We have now compared the susceptibility to NK lysis of cells infected with HCMV, strain AD169, and cells infected with a UL16 deletion mutant of this virus ([9]). In these experiments cells infected with the UL16 knockout virus are killed at substantially higher levels than cells infected with the wild-type virus. These data thus provide the first formal demonstration of an HCMV protein that can protect virus-infected cells from NK cell attack. The mechanism by which UL16 mediates this protection may be related to differences in the level of cell surface expression of NKG2D ligands observed between wild-type and knockout virus-infected cells. The increased susceptibility to NK cell lysis suffered by HCMVdeltaUL16 infected cells is not due to a differential expression of MHC class I on the target cell. Further experiments are now in progress to examine in more detail the mechanism by which UL16 sequesters, internalises or covers the ligands for NKG2D, in order to avoid NKG2D-mediated NK cell activation.

References:

1. Lilley, B. N., Ploegh, H. L. and Tirabassi, R. S., *J Virol* 2001. *75*: 11218-21.
2. Tortorella, D., Gewurz, B. E., Furman, M. H., Schust, D. J. and Ploegh, H. L., *Annu Rev Immunol* 2000. *18*: 861-926.
3. Cosman, D., Fanger, N. and Borges, L., *Immunol Rev* 1999. *168*: 177-85.
4. Tomasec, P., Braud, V. M., Rickards, C., Powell, M. B., McSharry, B. P., Gadola, S., Cerundolo, V., Borysiewicz, L. K., McMichael, A. J. and Wilkinson, G. W., *Science* 2000. *287*: 1031.
5. Sutherland, C. L., Chalupny, N. J. and Cosman, D., *Immunol Rev* 2001. *181*: 185-92.
6. Leong, C. C., Chapman, T. L., Bjorkman, P. J., Formankova, D., Mocarski, E. S., Phillips, J. H. and Lanier, L. L., *J. Exp. Med.* 1998. *187*: 1681-1687.
7. Cerboni, C., Mousavi-Jazi, M., Wakiguchi, H., Carbone, E., Karre, K. and Soderstrom, K., *Eur J Immunol* 2001. *31*: 2926-35.
8. Cosman, D., Mullberg, J., Sutherland, C. L., Chin, W., Armitage, R., Fanslow, W., Kubin, M. and Chalupny, N. J., *Immunity* 2001. *14*: 123-33.
9. Kaye, J., Browne, H., Stoffel, M. and Minson, T., *J Virol* 1992. *66*: 6609-15.

$CD8^+$ T cell immunodominance is multifactorial and is regulated by interferon-γ

Fernando Rodríguez[1], Stephanie Harkins[2], Mark Slifka[3] and J. Lindsay Whitton[2]

[1]Unidad de Investigación, Hospital 12 de Octubre, Madrid (Spain)
[2]Dept. Neuropharmacology, The Scripps Research Institute, La Jolla, CA. (USA)
[3]OHS J Vaccione and Gene Therapy Institute, Beaverton, OR. (USA)

Immunodominance occurs not only during microbial infections, but also following vaccination, reducing the potential diversity of memory T cells; this problem may be magnified when using subunit vaccines which, by definition, can induce a response to only a limited part of the microbial proteome. Therefore, it is important to identify the mechanisms which may regulate immunodominance; such an understanding may permit the rational design of vaccines which can circumvent the phenomenon, thereby inducing responses to both dominant and subdominant epitopes. Here, we show that immunodominance can be circumvented by the simple expedient of epitope separation and improvement of antigen presentation. DNA vaccines encoding isolated dominant and subdominant epitopes fused to ubiquitin to enhance their degradatrion in the proteasome, induce equivalent responses. Thus, a major component of immunodominance likely results from a defect in antigen presentation. In addition, we investigate the mechanism by which subdominant responses are suppressed, and we identify IFNγ as a key component. Mice lacking IFNγ mount markedly enhanced responses to normally-subdominant epitopes. Conversely, we show that priming of $CD8^+$-T cells to a single dominant epitope results in the strong suppression of responses to other normally-dominant sequences, in effect rendering these epitopes subdominant; this outcome depends entirely on IFNγ. Thus, for $CD8^+$-T cells, "original antigenic sin" results from the immunosuppressive effects of IFNγ, and this phenomenon is much less striking in mice lacking the cytokine.

Characterisation of ectromelia virus CrmD, a TNF binding protein

Margarida Saraiva and Antonio Alcamí

Division of Virology, Department of Pathology - University of Cambridge
Tennis Court Road - Cambridge CB2 1QP - UK

Poxviruses are large and complex DNA viruses that encode a group of molecules known to interfere with and evade the host immune system. The tumour necrosis factor (TNF) binding activity encoded by poxviruses is particularly interesting, since five different viral TNF receptor superfamily members have been described: cytokine response modifier (Crm) B, C, D and E, and a viral CD30 homologue. CrmB, C, D and E are known to interact with TNF or lymphotoxin while vCD30 interacts with the host CD30 ligand.

Ectromelia virus (EV) is the causative agent of mousepox and has been proposed as a model for the study of variola virus, which causes smallpox. In EV, crmB, C and E genes are truncated due to the presence of premature stop codons, while crmD and vCD30 are intact genes. In this study we characterised the EV encoded CrmD, showing that it is expressed at late times post-infection as a soluble TNF binding protein. We expressed this protein as a Fc fused protein and also, for the first time, in a mammalian system. We showed that CrmD specifically binds human, mouse or rat TNF, but not human lymphotoxin or seven other members of the TNF superfamily. The affinity for mouse TNF is higher than for human or rat, reinforcing EV as a mouse pathogen. We also showed that CrmD acts as a soluble decoy receptor, by blocking the binding of TNF to its cell surface receptors and inhibiting TNF-induced necrosis in a mouse cell line. Moreover, we also expressed different versions of CrmD in order to address the contribution of each region of the molecule to the binding of TNF, showing that the cysteine rich domains are sufficient for the binding of TNF, while the C-terminal region of the molecule is not needed.

We are now investigating the potential role of this C-terminal region of CrmD in immune evasion and looking for other possible ligands.

Mechanisms of cross-presentation during viral infections

Maria Carmen Ramirez, Anna L. Soukhanova and Luis J. Sigal

Fox Chase Cancer Center, Philadelphia PA

We have previously demonstrated that only bone marrow-derived professional antigen presenting cells (pAPC) can induce primary CTL responses to several viruses. We have also shown that when pAPC do not become infected with viruses, they can still initiate CTL responses by presenting viral antigens released by other infected cells (cross-presentation). Here we make use of a unique in vitro system to determine the types of pAPC that can cross-present viral antigens shed by virus infected cells, the pathways followed by these antigens, and the optimal characteristics of the antigenic proteins that determine efficient cross-presentation.

Significance of the viral interleukin-1β receptor in vaccinia vector immunization: *In vitro* and *in vivo* characterization of vaccinia virus MVA deletion mutants

Caroline Staib, and Gerd Sutter

GSF - Institut für Molekulare Virologie, 81675 München, Germany

Highly attenuated vaccinia virus MVA serves for development of candidate recombinant vaccines against infectious diseases and cancer (1). Several MVA vectors have already entered clinical evaluation. MVA was randomly obtained by long term tissue culture attenuation which resulted in great loss of genomic information including many genes regulating virus-host interactions. Nevertheless, some genes encoding poxviral immunomodulatory factors are maintained and their relevance for MVA-based vaccines remains to be determined.

The cytokine interleukin-1 (IL1) is an important regulator of inflammatory and immune responses that contribute to host defense against infection. Vaccinia virus encodes a viral soluble IL1β receptor (vIL1βR) which modulates acute phase host response to infection (induction of fever) and might influence induction of immune responses against virus-associated antigens (2). The gene encoding vIL1βR is conserved in the MVA genome and immunoprecipitation of an about 50 kDa protein using vIL1βR -specific antibodies confirmed its expression in MVA-infected cells. We obtained MVA mutant viruses defective in vIL1βR production (MVA-DvIL1βR) through transient insertion of selectable marker gene sequences which precisely deleted the vIL1βR coding sequences from the MVA genome (3). Analysis of MVA mutants indicated that deletion of the vIL1βR gene did not abrogate the formation of MVA progeny upon tissue culture propagation. After high dose intransal infection of mice with MVA-DvIL1βR, animals showed no signs of fever or other illness suggesting that the avirulent phenotype remained preserved for MVA-DvIL1βR. Results from ongoing experiments will be presented evaluating immunogenicity and protective capacity of MVA-DvIL1βR in comparison to non-mutated 1VA vaccines.

References:

1. Moss, B., M. W. Carroll, L. S. Wyatt, J. R. Bennink, V. M. Hirsch, S. Goldstein, W. R. Elkins, T. R. Fuerst, J. D. Lifson, M. Piatak, N. P. Restifo, W. Overwijk, R. Chamberlain, S. A. Rosenberg, and G. Sutter 1996. Host range restricted, non-replicating vaccinia virus vectors as vaccine candidates. Adv Exp Med Biol. 397:7-13.

2. Alcami, A., and G. Smith 1992. A soluble receptor for Interleukin-1b encoded by vaccinia virus: a novel mechanism of virus modulation of the host response to infection. Cell. 71:153-167.

3. Staib, C., I. Drexler, M. Ohlmann, S. Wintersperger, V. Erfle, and G. Sutter 2000. Transient host range selection for genetic engineering of modified vaccinia virus Ankara. Biotechniques. 28:1137-42, 1144-6, 1148.

The immunomodulatory genes m4, m6 and m152 of MCMV influence unequally the surface expression of different MHC I alleles

Markus Wagner

Using a recently established mutagenesis system for herpes viral bacterial artificial chromosomes, a set of MCMV mutants with exact single, double and triple deletions of the known MHC class I inter-ac-ting genes m04, m06 and m152 has been generated. In contrast to previous investigations, these set of MCMV mutants allowed for the first time investigation of the relative importance of each individual gene for MHC class I modulation in the context of viral infection. We tested the effect of the mutants on down regulation of the MHC class I alleles Kd, Dd, Ld, Kb, Db, Kk and Lq on infected cells 4 to 16 hours p.i. We could show that:

· the virus mutant lacking m04, m06 and m152 expression does not induce a MHC class I allele retention after infection, suggesting that there are no other MHC class I modulatory genes encoded by MCMV.

· certain MHC I alleles are more affected by the viral proteins than others. The Kb allele is only slightly down regulated after wt MCMV infection, whereas Kd, Dd, Db and Lq show a residual surface expression of only about 10%.

· deletion of m06/gp48 alone fully restores MHC class I surface expression in case of Kd, Dd, Ld, Kb and Kk, and at least increase it for Db and Lq. Deletion of m152/gp40 has a minor effect, showing that m06/gp48 is the dominant MHC class I down regulating gene during MCMV infection.

· m06/gp48 and m152/gp40 in combination are more efficient in MHC class I down regulation than each individual gene alone.

· A mutant expressing only gp34 has as little effect on MHC class I as the mutant lacking all three genes, showing that m04/gp34 does not actively affect MHC class I surface expression. However, in presence of m06 or m152, the m04 product rescues MHC class I surface expression and antagonizes the function of m06/gp48 and m152/gp40 with respect to MHC class I surface expression.

The viral chemokine binding protein M3 interacts with both the heparin-binding domain and N-terminal loop of IL-8

Louise M.C. Webb, Ian Clark-Lewis, and Antonio Alcami

An effective immune response requires the extravasation of leukocytes from blood to the site of infection. Chemokines are small heparin-binding proteins that direct migration of leukocytes by signalling through a family of G protein-coupled receptors. The central importance of chemokines in physiology is underscored by the fact that many viruses encode proteins that disrupt the chemokine system. The murine gammaherpesvirus-68 gene M3 encodes a chemokine binding protein. Initial characterisation of M3 showed that it binds to a broad range of chemokines and inhibits both receptor binding and chemokine-induced calcium influx (1).

We have used a panel of IL-8 analogs to investigate the interaction of IL-8 with M3. By using the scintillation proximity assay, we show that the N–terminal loop and C-terminal heparin-binding domain of IL-8 are important for M3 binding. Furthermore, we show that M3 can prevent IL-8 binding to heparin and displace heparin-bound IL-8. However, chemotaxis induced by an analog of IL-8 which lacks the entire heparin-binding domain is still inhibited by M3. Together, this data implies that the M3 protein uses the N-terminal loop of IL-8 to inhibit receptor binding but is also able to disrupt the chemokine-glycosaminoglycan interaction. This feature of M3 could allow it to prevent the formation of chemokine gradients established at sites of infection in addition to neutralising chemokine activity.

References:

(1). A Braod Spectrum Secreted Chemokine Binding Protein Encoded by a Herpesvirus. C.M. Parry, J.P. Simas, V.P.Smith, C.A.Steward, A.C.Minson, S. Efstathiou, and A.Alcami. J. Exp. Med. 191: 573-578. 2000.

LIST OF INVITED SPEAKERS

Antonio Alcami — Depts. of Medicine and Pathology, University of Cambridge. Hills Road, Cambridge CB2 2QQ (UK). Tel.: 44 1223 76 34 03. Fax: 44 1223 33 68 46. E-mail: aa258@mole.bio.cam. ac.uk

Chris Boshoff — Wolfson Institute for Biomedical Research, Cruciform Building. Gower Street. University College London, WC1 E6AE London (UK). Tel.: 44 20 7679 6850. Fax: 44 20 7679 6851. E-mail: c.boshoff@ucl.ac.uk

Margarita Del Val — Centro Nacional de Biología Fundamental. Instituto de Salud Carlos III. Ctra. Pozuelo, Km.2, 28220 Madrid (Spain). Tel.: 34 91 509 79 43. Fax: 34 91 509 79 19. E-mail: mdval@isciii.es

Mariano Esteban — Centro Nacional de Biotecnología, Campus de la Univ. Autónoma de Madrid. Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 585 45 03. Fax: 34 91 585 45 06. E-mail: mesteban@cnb.uam.es

Peter Ghazal — Centre for Genomic Technology& Informatics. Univ. of Edinburgh. Summerhall, EH9 1QH Edinburgh (UK). Tel.: 44 131 650 7840. Fax: 44 131 650 6511. E-mail: p.ghazal@ ed.ac.uk

Hans Hengartner — Experimental Immunology, University and ETH Zürich. Schmelzbergstr. 12, 8091 Zurich (Switzerland). Tel.: 41 1 255 29 89. Fax: 41 1 255 44 20. E-mail: has.hengartner@ pty.usz.ch

David C. Johnson — Department of Molecular Microbiology & Immunology, Oregon Health Sciences University, Portland, OR. 97201 (USA). Tel.: 1 503 494 0834. Fax: 1 503 494 6862. E-mail: johnsoda@ohsu.edu

Ulrich H. Koszinowski — Max von Pettenkofer-Institut. Pettenkoferstraße 9a, 80336 München (Germany). Tel.: 49 89 51 60 52 90. Fax: 49 89 51 60 52 92. E-mail: koszinowski@m3401.mpk.med.uni-muenchen.de

Sergio A. Lira — Schering-Plough Research Institute. 2015 Galloping Hill Rd, Kenilworth, NJ. 07033 (USA). Tel.: 1 908 740 30 88. Fax: 1 908 740 30 84. E-mail: sergio.lira@spcorp.com

Carlos Martínez-A.	Dept. of Immunology & Oncology, Centro Nacional de Biotecnología/CSIC. UAM Campus de Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 585 45 37. Fax: 34 91 372 04 93. E-mail: cmartineza@cnb.uam.es
Grant McFadden	Dept. of Microbiology and Immunology. The Univ. of Western Ontario and The John P. Robarts Research Inst. London. 1400 Western Road, N6G 2V4 Ontario (Canada). Tel.: 1 519 663 31 84. Fax: 1 519 663 38 47. E-mail: mcfadden@rri.ca
Andrew J. McMichael	MRC Human Immunology Unit. John Radcliffe Hospital, Oxford OX3 9UT (UK). Tel.: 44 1865 222 336. Fax: 44 1865 222 600. E-mail: andrew.mcmichael@clinical-medicine.oxford.ac.uk
Edward S. Mocarski	Department of Microbiology & Immunology, Standford University. 299 Campus Dr., Standford, CA. 94305-5124 (USA). Tel.: 1 650 723 64 35. Fax: 1 650 723 16 06. E-mail: mocarski@stanford.edu
Bernard Moss	Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health. 4 Center Drive, Bethesda, Maryland, 20892-0445 (USA). Tel.: 1 301 496 9869. Fax: 1 301 480 1147. E-mail: bmoss@nih.gov
Anthony A. Nash	Department of Veterinary Pathology, Univ.of Edinburgh. Summerhall, EH9 1QH Edinburgh (UK). Tel.: 44 131 650 61 64. Fax: 44 131 650 65 11. E-mail: aanash@ vet.ed.ac.uk
Hidde L. Ploegh	Department of Pathology. Harvard Medical School. 200 Longwood Ave, Boston, MA. 02115 (USA). Tel.: 1 617 432 47 76. Fax: 1 617 432 47 75. E-mail: ploegh@hms.harvard.edu
Anthony A. Scalzo	Department of Microbiology, Univ. of Western Australia, Nedlands, WA, 6009 (Australia). Tel.: 61 8 9346 2058. Fax: 61 8 9346 2912. E-mail: scals@cyllene.uwa.edu.au
Geoffrey L. Smith	The Wright-Fleming Institute, Faculty of Medicine, Imperial College. St. Mary's Campus. Norfolk Place, London W2 1PG (UK). Tel.: 44 207 594 3972. Fax: 44 207 594 3973
Jonathan W. Yewdell	Cellular Biology Section Laboratory of Viral Diseases. National Institute of Allergy and Infectious Diseases. Center Drive, Bethesda, MD. 20892-0440 (USA). Fax: 1 301 402 7362. E-mail: JYEWDELL@ niaid.nih.gov

LIST OF PARTICIPANTS

Covadonga Alonso	Dpto. Biotecnología, INIA. Ctra. La Coruña Km. 7.5, 28040 Madrid (Spain). Tel.: 34 91 347 68 96. Fax: 34 91 357 22 93. E-mail: calonso@inia.es
Ana Angulo	Institut d'Investigacions Biomediques Pi y Sunyer. Villarroel 170, 08036 Barcelona (Spain). Tel.: 34 93 403 52 86. E-mail: aangulo@medicina.ub.es
Luis C. Antón	Centro de Biología Molecular "Severo Ochoa". Universidad Autónoma de Madrid. Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 397 80 40. Fax: 34 91 397 80 87. E-mail: lanton@cbm.uam.es
Elaine Bell	Nature Reviews Immunology. The MacMillan Building. 4 Crinan Street, London N1 9XW (UK). Tel.: 44 20 7843 4992. Fax: 44 20 7843 3629. E-mail: e.bell@nature.com
Rafael Blasco	Dpto. Biotecnología, INIA. Ctra. La Coruña Km 7.5, 28040 Madrid (Spain). Tel.: 34 91 347 39 13. Fax: 34 91 357 22 93. E-mail: blasco@inia.es
Hans-Gerhard Burgert	Max-von-Pettenkofer Institute, Gene Center, Ludwig-Maximilians-University. Feodor-Lynen-Str. 25, 81377 Munich (Germany). Tel.: 49 2180 68 52. Fax: 49 2180 68 99. E-mail: burgert@lmb.uni-muenchen.de
Jose Casasnovas	Department of Biosciences. Karolinska Institute. NOVUM, Center for Biotechnology. Halsovagen 7, 141 57 Huddinge (Sweden). Tel.: 46 8 608 91 33. Fax: 46 8 774 55 38. E-mail: jose.casasnovas@cbt.ki.se
Raúl Castaño	Dept. Immunology. Hospital de la Princesa. Diego de León 62, 28006 Madrid (Spain). Tel.: 34 91 520 23 70. Fax: 34 91 520 23 74. E-mail: rcastano@hlpr.insalud.es
Alberto Fraile-Ramos	MRC Laboratory for Molecular Cell Biology. University College London. Gower Street, London WC1E 6BT (UK). Tel.: 44 20 7679 3543. Fax: 44 20 7679 7805. E-mail: dmcbafr@ucl.ac.uk
Begoña Galocha	Hospital Clínico San Carlos. Ciudad Universitaria, 28040 Madrid (Spain). Tel.: 34 91 330 34 34. Fax: 34 91 330 36 15. E-mail: bgalocha@hcsc.es
Hartmut Hengel	Robert Koch-Institut, Fachgebiet Virale Infektionen, 13353 Berlin (Germany). Tel.: 49 1888 754 2502. Fax: 49 1888 754 2328. E-mail: hengelh@rki.de

Ann B. Hill	Dept. of Molecular Mircrobiology and Immunology. Oregon Health Sciences University L220. 3181 SW Sam Jackson Park Rd, Portland, OR. 97201 (USA). Tel.: 1 503 494 0763. Fax: 1 503 494 6862. E-mail: hillan@ohsu.edu
Stipan Jonjic	Faculty of Medicine. Brace Branchetta 20, 51000 Rijeka (Croatia). Tel.: 385 51 651 170. Fax: 385 51 651 176. E-mail: jstipan@medri.hr
Gunasegaran Karupiah	Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University. PO Box 334, Canberra, ACT 2601 (AUSTRALIA). Tel.: 61 2 6125 4562. Fax: 61 2 6125 2595. E-mail: Guna.Karupiah @anu.edu.au
Daniel López	Centro Nacional de Biología Fundamental. Instituto de Salud Carlos III. Ctra. Pozuelo Km. 2, 28220 Majadahonda, Madrid (Spain). Tel.: 34 91 509 70 61. Fax: 34 91 509 79 19. E-mail: dlopez@isciii.es
José A. López de Castro	Centro de Biología Molecular"Severo Ochoa". Universidad Autónoma de Madrid. Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 397 80 50. Fax: 34 91 397 80 87. E-mail: aldecastro@cbm.uam.es
Miguel López-Botet	DCEXS (Inmunología). Univ. Pompeu Fabra. Dr. Aiguader 80, 08003 Barcelona (Spain). Tel.: 34 93 542 28 47. Fax: 34 93 542 28 02. E-mail: miguel.lopez-botet@cexs.upf.es
José A. Melero	Centro Nacional de Biología Fundamental, Instituto de Salud Carlos III. Ctra. Pozuelo Km 2, Majadahonda, Madrid (Spain). Tel.: 34 91 509 7941. Fax: 34 91 509 7919. E-mail: jmelero@isciii.es
Pilar Najarro	Wright Fleming Institute, Imperial College of Science, Technology and Medicine. St. Mary's Campus, Norfolk Place, London, W2 1PG (UK). Tel.: 44 207 594 39 80. Fax: 44 207 594 39 73. E-mail: p.najarro@ic.ac.uk
Marta Oteo	Centro Nacional de Biología Fundamental. Instituto de Salud Carlos III. Ctra. Pozuelo, Km.2, 28220 Madrid (Spain). Tel.: 34 91 509 79 01. Fax: 34 91 509 79 19. E-mail: moteo@isciii.es
Susan R. Owens	European Molecular Biology Organisation. Meyerhofstrasse 1, 69117 Heidelberg (Germany). Tel.: 49 6221 8891 305. Fax: 49 6221 8891 230. E-mail: susan.owens@embo.org
Florian Puehler	Dept. Virology. Inst. for Medical Microbiology and Hygiene. University of Freiburg. Hermann-Herder-Str. 11, 79104 Freiburg (Germany). Tel.: 49 761 203 6622. Fax: 49 761 203 6562. E-mail: puehler@ukl.uni-freiburg.de

Hugh Reyburn	Dept. of Pathology, Univ. of Cambridge. Tennis Court Road, Cambridge CB2 1QP (UK). Tel.: 44 1223 766 422. Fax: 44 1223 333 875. E-mail: htr20@ hermes.cam.ac.uk
Fernando Rodríguez	Unidad de Investigación, Hospital 12 de Octubre, 28041 Madrid (Spain). Tel.: 34 91 390 84 11. Fax: 34 91 390 85 44. E-mail: fernandor@h12o.es
Margarida Saraiva	Division of Virology, Dept. of Pathology-University of Cambridge. Tennis Court Road, Cambridge CB2 1QP (UK). Tel.: 44 1223 336922. Fax: 44 1223 336926. E-mail: msdsss2@mole.bio.cam.ac.uk
Günther Schönrich	Institute of Virology. Charité Medical School. Humboldt University Berlin. Schumannstr. 20/21, 10117 Berlin (Germany). Tel.: 49 30 450 525 907. Fax: 49 30 450 525 071. E-mail: guenther.schoenrich@charite.de
Luis J. Sigal	Fox Chase Cancer Center. 7701 Burholme Avenue, Philadelphia PA. 19111 (USA). Tel.: 1 215 728 7061. Fax: 1 413 487 5406. E-mail: LJ_Sigal@fccc.edu
Gerd Sutter	GSF - Institut für Molekulare Virologie. Trogerstr. 4b, 81675 München (Germany). Tel.: 49 89 41 40 74 43. Fax: 49 89 41 40 74 44. E-mail: sutter@gsf.de
Markus Wagner	Department of Virology. Max von Pettenkofer Institut. Pettenkoferstrasse 9a, 80336 München (Germany). Tel.: 49 89 51 60 52 52. Fax: 49 89 51 60 52 92. E-mail: wagner@ lmb.uni-muenchen.de
Louise M. C. Webb	Division of Virology. Dept. of Pathology. University of Cambridge. Tennis Court Road, Cambridge CB2 1QP (UK). Tel.: 44 1223 33 69 22. Fax: 44 1223 33 69 26. E-mail: lmcw2@mole.bio.cam.ac.uk
Jamie D. K. Wilson	Nature Immunology. 345, Park Avenue South, New York, NY. 10010-1707 (USA). Tel.: 1 212 726 92 07. Fax: 1 212 696 97 52. E-mail: j.wilson@NATURENY.com

Texts published in the
SERIE UNIVERSITARIA
by the
FUNDACIÓN JUAN MARCH
concerning workshops and courses organized within the
Plan for International Meetings on Biology (1989-1991)

---

*: Out of stock.

*246 **Workshop on Tolerance: Mechanisms and Implications.**
Organizers: P. Marrack and C. Martínez-A.

*247 **Workshop on Pathogenesis-related Proteins in Plants.**
Organizers: V. Conejero and L. C. Van Loon.

*248 **Course on DNA - Protein Interaction.**
M. Beato.

*249 **Workshop on Molecular Diagnosis of Cancer.**
Organizers: M. Perucho and P. García Barreno.

*251 **Lecture Course on Approaches to Plant Development.**
Organizers: P. Puigdomènech and T. Nelson.

*252 **Curso Experimental de Electroforesis Bidimensional de Alta Resolución.**
Organizer: Juan F. Santarén.

253 **Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.**
Organizers: F. García-Arenal and P. Palukaitis.

254 **Advanced Course on Biochemistry and Genetics of Yeast.**
Organizers: C. Gancedo, J. M. Gancedo, M. A. Delgado and I. L. Calderón.

*255 **Workshop on the Reference Points in Evolution.**
Organizers: P. Alberch and G. A. Dover.

*256 **Workshop on Chromatin Structure and Gene Expression.**
Organizers: F. Azorín, M. Beato and A. A. Travers.

257 **Lecture Course on Polyamines as Modulators of Plant Development.**
Organizers: A. W. Galston and A. F. Tiburcio.

*258 **Workshop on Flower Development.**
Organizers: H. Saedler, J. P. Beltrán and J. Paz-Ares.

*259 **Workshop on Transcription and Replication of Negative Strand RNA Viruses.**
Organizers: D. Kolakofsky and J. Ortín.

*260 **Lecture Course on Molecular Biology of the Rhizobium-Legume Symbiosis.**
Organizer: T. Ruiz-Argüeso.

261 **Workshop on Regulation of Translation in Animal Virus-Infected Cells.**
Organizers: N. Sonenberg and L. Carrasco.

*263 **Lecture Course on the Polymerase Chain Reaction.**
Organizers: M. Perucho and E. Martínez-Salas.

*264 **Workshop on Yeast Transport and Energetics.**
Organizers: A. Rodríguez-Navarro and R. Lagunas.

265 **Workshop on Adhesion Receptors in the Immune System.**
Organizers: T. A. Springer and F. Sánchez-Madrid.

*266 **Workshop on Innovations in Proteases and Their Inhibitors: Fundamental and Applied Aspects.**
Organizer: F. X. Avilés.

267 **Workshop on Role of Glycosyl-Phosphatidylinositol in Cell Signalling.**
Organizers: J. M. Mato and J. Larner.

268 **Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects.**
Organizers: R. Serrano and J. A. Pintor-Toro.

269 **Workshop on Neural Control of Movement in Vertebrates.**
Organizers: R. Baker and J. M. Delgado-García.

Texts published by the CENTRE FOR INTERNATIONAL MEETINGS ON BIOLOGY

1 **Workshop on What do Nociceptors Tell the Brain?**
Organizers: C. Belmonte and F. Cerveró.

*2 **Workshop on DNA Structure and Protein Recognition.**
Organizers: A. Klug and J. A. Subirana.

*3 **Lecture Course on Palaeobiology: Preparing for the Twenty-First Century.**
Organizers: F. Álvarez and S. Conway Morris.

*4 **Workshop on the Past and the Future of Zea Mays.**
Organizers: B. Burr, L. Herrera-Estrella and P. Puigdomènech.

*5 **Workshop on Structure of the Major Histocompatibility Complex.**
Organizers: A. Arnaiz-Villena and P. Parham.

*6 **Workshop on Behavioural Mechanisms in Evolutionary Perspective.**
Organizers: P. Bateson and M. Gomendio.

*7 **Workshop on Transcription Initiation in Prokaryotes**
Organizers: M. Salas and L. B. Rothman-Denes.

*8 **Workshop on the Diversity of the Immunoglobulin Superfamily.**
Organizers: A. N. Barclay and J. Vives.

9 **Workshop on Control of Gene Expression in Yeast.**
Organizers: C. Gancedo and J. M. Gancedo.

*10 **Workshop on Engineering Plants Against Pests and Pathogens.**
Organizers: G. Bruening, F. García-Olmedo and F. Ponz.

11 **Lecture Course on Conservation and Use of Genetic Resources.**
Organizers: N. Jouve and M. Pérez de la Vega.

12 **Workshop on Reverse Genetics of Negative Stranded RNA Viruses.**
Organizers: G. W. Wertz and J. A. Melero.

*13 **Workshop on Approaches to Plant Hormone Action**
Organizers: J. Carbonell and R. L. Jones.

*14 **Workshop on Frontiers of Alzheimer Disease.**
Organizers: B. Frangione and J. Ávila.

*15 **Workshop on Signal Transduction by Growth Factor Receptors with Tyrosine Kinase Activity.**
Organizers: J. M. Mato and A. Ullrich.

16 **Workshop on Intra- and Extra-Cellular Signalling in Hematopoiesis.**
Organizers: E. Donnall Thomas and A. Grañena.

*17 **Workshop on Cell Recognition During Neuronal Development.**
Organizers: C. S. Goodman and F. Jiménez.

18 **Workshop on Molecular Mechanisms of Macrophage Activation.**
Organizers: C. Nathan and A. Celada.

*19 **Workshop on Viral Evasion of Host Defense Mechanisms.**
Organizers: M. B. Mathews and M. Esteban.

*20 **Workshop on Genomic Fingerprinting.**
Organizers: M. McClelland and X. Estivill.

21 **Workshop on DNA-Drug Interactions.**
Organizers: K. R. Fox and J. Portugal.

*22 **Workshop on Molecular Bases of Ion Channel Function.**
Organizers: R. W. Aldrich and J. López-Barneo.

*23 **Workshop on Molecular Biology and Ecology of Gene Transfer and Propagation Promoted by Plasmids.**
Organizers: C. M. Thomas, E. M. H. Willington, M. Espinosa and R. Díaz Orejas.

*24 **Workshop on Deterioration, Stability and Regeneration of the Brain During Normal Aging.**
Organizers: P. D. Coleman, F. Mora and M. Nieto-Sampedro.

25 **Workshop on Genetic Recombination and Defective Interfering Particles in RNA Viruses.**
Organizers: J. J. Bujarski, S. Schlesinger and J. Romero.

26 **Workshop on Cellular Interactions in the Early Development of the Nervous System of Drosophila.**
Organizers: J. Modolell and P. Simpson.

*27 **Workshop on Ras, Differentiation and Development.**
Organizers: J. Downward, E. Santos and D. Martín-Zanca.

*28 **Workshop on Human and Experimental Skin Carcinogenesis.**
Organizers: A. J. P. Klein-Szanto and M. Quintanilla.

*29 **Workshop on the Biochemistry and Regulation of Programmed Cell Death.**
Organizers: J. A. Cidlowski, R. H. Horvitz, A. López-Rivas and C. Martínez-A.

*30 **Workshop on Resistance to Viral Infection.**
Organizers: L. Enjuanes and M. M. C. Lai.

31 **Workshop on Roles of Growth and Cell Survival Factors in Vertebrate Development.**
Organizers: M. C. Raff and F. de Pablo.

32 **Workshop on Chromatin Structure and Gene Expression.**
Organizers: F. Azorín, M. Beato and A. P. Wolffe.

*33 **Workshop on Molecular Mechanisms of Synaptic Function.**
Organizers: J. Lerma and P. H. Seeburg.

*34 **Workshop on Computational Approaches in the Analysis and Engineering of Proteins.**
Organizers: F. S. Avilés, M. Billeter and E. Querol.

35 **Workshop on Signal Transduction Pathways Essential for Yeast Morphogenesis and Cell Integrity.**
Organizers: M. Snyder and C. Nombela.

36 **Workshop on Flower Development.**
Organizers: E. Coen, Zs. Schwarz-Sommer and J. P. Beltrán.

*37 **Workshop on Cellular and Molecular Mechanism in Behaviour.**
Organizers: M. Heisenberg and A. Ferrús.

38 **Workshop on Immunodeficiencies of Genetic Origin.**
Organizers: A. Fischer and A. Arnaiz-Villena.

39 **Workshop on Molecular Basis for Biodegradation of Pollutants.**
Organizers: K. N. Timmis and J. L. Ramos.

*40 **Workshop on Nuclear Oncogenes and Transcription Factors in Hematopoietic Cells.**
Organizers: J. León and R. Eisenman.

*41 **Workshop on Three-Dimensional Structure of Biological Macromolecules.**
Organizers: T. L Blundell, M. Martínez-Ripoll, M. Rico and J. M. Mato.

42 **Workshop on Structure, Function and Controls in Microbial Division.**
Organizers: M. Vicente, L. Rothfield and J. A. Ayala.

*43 **Workshop on Molecular Biology and Pathophysiology of Nitric Oxide.**
Organizers: S. Lamas and T. Michel.

*44 **Workshop on Selective Gene Activation by Cell Type Specific Transcription Factors.**
Organizers: M. Karin, R. Di Lauro, P. Santisteban and J. L. Castrillo.

45 **Workshop on NK Cell Receptors and Recognition of the Major Histocompatibility Complex Antigens.**
Organizers: J. Strominger, L. Moretta and M. López-Botet.

46 **Workshop on Molecular Mechanisms Involved in Epithelial Cell Differentiation.**
Organizers: H. Beug, A. Zweibaum and F. X. Real.

47 **Workshop on Switching Transcription in Development.**
Organizers: B. Lewin, M. Beato and J. Modolell.

48 **Workshop on G-Proteins: Structural Features and Their Involvement in the Regulation of Cell Growth.**
Organizers: B. F. C. Clark and J. C. Lacal.

*49 **Workshop on Transcriptional Regulation at a Distance.**
Organizers: W. Schaffner, V. de Lorenzo and J. Pérez-Martín.

50 **Workshop on From Transcript to Protein: mRNA Processing, Transport and Translation.**
Organizers: I. W. Mattaj, J. Ortín and J. Valcárcel.

51 **Workshop on Mechanisms of Expression and Function of MHC Class II Molecules.**
Organizers: B. Mach and A. Celada.

52 **Workshop on Enzymology of DNA-Strand Transfer Mechanisms.**
Organizers: E. Lanka and F. de la Cruz.

53 **Workshop on Vascular Endothelium and Regulation of Leukocyte Traffic.**
Organizers: T. A. Springer and M. O. de Landázuri.

54 **Workshop on Cytokines in Infectious Diseases.**
Organizers: A. Sher, M. Fresno and L. Rivas.

55 **Workshop on Molecular Biology of Skin and Skin Diseases.**
Organizers: D. R. Roop and J. L. Jorcano.

56 **Workshop on Programmed Cell Death in the Developing Nervous System.**
Organizers: R. W. Oppenheim, E. M. Johnson and J. X. Comella.

57 **Workshop on NF-κB/IκB Proteins. Their Role in Cell Growth, Differentiation and Development.**
Organizers: R. Bravo and P. S. Lazo.

58 **Workshop on Chromosome Behaviour: The Structure and Function of Telomeres and Centromeres.**
Organizers: B. J. Trask, C. Tyler-Smith, F. Azorín and A. Villasante.

59 **Workshop on RNA Viral Quasispecies.**
Organizers: S. Wain-Hobson, E. Domingo and C. López Galíndez.

60 **Workshop on Abscisic Acid Signal Transduction in Plants.**
Organizers: R. S. Quatrano and M. Pagès.

61 **Workshop on Oxygen Regulation of Ion Channels and Gene Expression.**
Organizers: E. K. Weir and J. López-Barneo.

62 **1996 Annual Report**

63 **Workshop on TGF-β Signalling in Development and Cell Cycle Control.**
Organizers: J. Massagué and C. Bernabéu.

64 **Workshop on Novel Biocatalysts.**
Organizers: S. J. Benkovic and A. Ballesteros.

65 **Workshop on Signal Transduction in Neuronal Development and Recognition.**
Organizers: M. Barbacid and D. Pulido.

66 **Workshop on 100th Meeting: Biology at the Edge of the Next Century.**
Organizer: Centre for International Meetings on Biology, Madrid.

67 **Workshop on Membrane Fusion.**
Organizers: V. Malhotra and A. Velasco.

68 **Workshop on DNA Repair and Genome Instability.**
Organizers: T. Lindahl and C. Pueyo.

69 **Advanced course on Biochemistry and Molecular Biology of Non-Conventional Yeasts.**
Organizers: C. Gancedo, J. M. Siverio and J. M. Cregg.

70 **Workshop on Principles of Neural Integration.**
Organizers: C. D. Gilbert, G. Gasic and C. Acuña.

71 **Workshop on Programmed Gene Rearrangement: Site-Specific Recombination.**
Organizers: J. C. Alonso and N. D. F. Grindley.

72 **Workshop on Plant Morphogenesis.**
Organizers: M. Van Montagu and J. L. Micol.

73 **Workshop on Development and Evolution.**
Organizers: G. Morata and W. J. Gehring.

*74 **Workshop on Plant Viroids and Viroid-Like Satellite RNAs from Plants, Animals and Fungi.**
Organizers: R. Flores and H. L. Sänger.

75 **1997 Annual Report.**

76 **Workshop on Initiation of Replication in Prokaryotic Extrachromosomal Elements.**
Organizers: M. Espinosa, R. Díaz-Orejas, D. K. Chattoraj and E. G. H. Wagner.

77 **Workshop on Mechanisms Involved in Visual Perception.**
Organizers: J. Cudeiro and A. M. Sillito.

78 **Workshop on Notch/Lin-12 Signalling.**
Organizers: A. Martínez Arias, J. Modolell and S. Campuzano.

79 **Workshop on Membrane Protein Insertion, Folding and Dynamics.**
Organizers: J. L. R. Arrondo, F. M. Goñi, B. De Kruijff and B. A. Wallace.

80 **Workshop on Plasmodesmata and Transport of Plant Viruses and Plant Macromolecules.**
Organizers: F. García-Arenal, K. J. Oparka and P.Palukaitis.

81 **Workshop on Cellular Regulatory Mechanisms: Choices, Time and Space.**
Organizers: P. Nurse and S. Moreno.

82 **Workshop on Wiring the Brain: Mechanisms that Control the Generation of Neural Specificity.**
Organizers: C. S. Goodman and R. Gallego.

83 **Workshop on Bacterial Transcription Factors Involved in Global Regulation.**
Organizers: A. Ishihama, R. Kolter and M. Vicente.

84 **Workshop on Nitric Oxide: From Discovery to the Clinic.**
Organizers: S. Moncada and S. Lamas.

85 **Workshop on Chromatin and DNA Modification: Plant Gene Expression and Silencing.**
Organizers: T. C. Hall, A. P. Wolffe, R. J. Ferl and M. A. Vega-Palas.

86 **Workshop on Transcription Factors in Lymphocyte Development and Function.**
Organizers: J. M. Redondo, P. Matthias and S. Pettersson.

87 **Workshop on Novel Approaches to Study Plant Growth Factors.**
Organizers: J. Schell and A. F. Tiburcio.

88 **Workshop on Structure and Mechanisms of Ion Channels.**
Organizers: J. Lerma, N. Unwin and R. MacKinnon.

89 **Workshop on Protein Folding.**
Organizers: A. R. Fersht, M. Rico and L. Serrano.

90 **1998 Annual Report.**

91 **Workshop on Eukaryotic Antibiotic Peptides.**
Organizers: J. A. Hoffmann, F. García-Olmedo and L. Rivas.

92 **Workshop on Regulation of Protein Synthesis in Eukaryotes.**
Organizers: M. W. Hentze, N. Sonenberg and C. de Haro.

93 **Workshop on Cell Cycle Regulation and Cytoskeleton in Plants.**
Organizers: N.-H. Chua and C. Gutiérrez.

94 **Workshop on Mechanisms of Homologous Recombination and Genetic Rearrangements.**
Organizers: J. C. Alonso, J. Casadesús, S. Kowalczykowski and S. C. West.

95 **Workshop on Neutrophil Development and Function.**
Organizers: F. Mollinedo and L. A. Boxer.

96 **Workshop on Molecular Clocks.**
Organizers: P. Sassone-Corsi and J. R. Naranjo.

97 **Workshop on Molecular Nature of the Gastrula Organizing Center: 75 years after Spemann and Mangold.**
Organizers: E. M. De Robertis and J. Aréchaga.

98 **Workshop on Telomeres and Telomerase: Cancer, Aging and Genetic Instability.**
Organizer: M. A. Blasco.

99 **Workshop on Specificity in Ras and Rho-Mediated Signalling Events.**
Organizers: J. L. Bos, J. C. Lacal and A. Hall.

100 **Workshop on the Interface Between Transcription and DNA Repair, Recombination and Chromatin Remodelling.**
Organizers: A. Aguilera and J. H. J. Hoeijmakers.

101 **Workshop on Dynamics of the Plant Extracellular Matrix.**
Organizers: K. Roberts and P. Vera.

102 **Workshop on Helicases as Molecular Motors in Nucleic Acid Strand Separation.**
Organizers: E. Lanka and J. M. Carazo.

103 **Workshop on the Neural Mechanisms of Addiction.**
Organizers: R. C. Malenka, E. J. Nestler and F. Rodríguez de Fonseca.

104 **1999 Annual Report.**

105 **Workshop on the Molecules of Pain: Molecular Approaches to Pain Research.**
Organizers: F. Cervero and S. P. Hunt.

106 **Workshop on Control of Signalling by Protein Phosphorylation.**
Organizers: J. Schlessinger, G. Thomas, F. de Pablo and J. Moscat.

107 **Workshop on Biochemistry and Molecular Biology of Gibberellins.**
Organizers: P. Hedden and J. L. García-Martínez.

108 **Workshop on Integration of Transcriptional Regulation and Chromatin Structure.**
Organizers: J. T. Kadonaga, J. Ausió and E. Palacián.

109 **Workshop on Tumor Suppressor Networks.**
Organizers: J. Massagué and M. Serrano.

110 **Workshop on Regulated Exocytosis and the Vesicle Cycle.**
Organizers: R. D. Burgoyne and G. Álvarez de Toledo.

111 **Workshop on Dendrites.**
Organizers: R. Yuste and S. A. Siegelbaum.

112 **Workshop on the Myc Network: Regulation of Cell Proliferation, Differentiation and Death.**
Organizers: R. N. Eisenman and J. León.

113 **Workshop on Regulation of Messenger RNA Processing.**
Organizers: W. Keller, J. Ortín and J. Valcárcel.

114 **Workshop on Genetic Factors that Control Cell Birth, Cell Allocation and Migration in the Developing Forebrain.**
Organizers: P. Rakic, E. Soriano and A. Álvarez-Buylla.

115 **Workshop on Chaperonins: Structure and Function.**
Organizers: W. Baumeister, J. L. Carrascosa and J. M. Valpuesta.

116 **Workshop on Mechanisms of Cellular Vesicle and Viral Membrane Fusion.**
Organizers: J. J. Skehel and J. A. Melero.

117 **Workshop on Molecular Approaches to Tuberculosis.**
Organizers: B. Gicquel and C. Martín.

118 **2000 Annual Report.**

119 **Workshop on Pumps, Channels and Transporters: Structure and Function.**
Organizers: D. R. Madden, W. Kühlbrandt and R. Serrano.

120 **Workshop on Common Molecules in Development and Carcinogenesis.**
Organizers: M. Takeichi and M. A. Nieto.

121 **Workshop on Structural Genomics and Bioinformatics.**
Organizers: B. Honig, B. Rost and A. Valencia.

122 **Workshop on Mechanisms of DNA-Bound Proteins in Prokaryotes.**
Organizers: R. Schleif, M. Coll and G. del Solar.

123 **Workshop on Regulation of Protein Function by Nitric Oxide.**
Organizers: J. S. Stamler, J. M. Mato and S. Lamas.

124 **Workshop on the Regulation of Chromatin Function.**
Organizers: F. Azorín, V. G. Corces, T. Kouzarides and C. L. Peterson.

125 **Workshop on Left-Right Asymmetry.**
Organizers: C. J. Tabin and J. C. Izpisúa Belmonte.

126 **Workshop on Neural Prepatterning and Specification.**
Organizers: K. G. Storey and J. Modolell.

127 **Workshop on Signalling at the Growth Cone.**
Organizers: E. R. Macagno, P. Bovolenta and A. Ferrús.

128 **Workshop on Molecular Basis of Ionic Homeostasis and Salt Tolerance in Plants.**
Organizers: E. Blumwald and A. Rodríguez-Navarro.

129 **Workshop on Cross Talk Between Cell Division Cycle and Development in Plants.**
Organizers: V. Sundaresan and C. Gutiérrez.

130 **Workshop on Molecular Basis of Human Congenital Lymphocyte Disorders.**
Organizers: H. D. Ochs and J. R. Regueiro.

131 **Workshop on Genomic vs Non-Genomic Steroid Actions: Encountered or Unified Views.**
Organizers: M. G. Parker and M. A. Valverde.

132 **2001 Annual Report.**

133 **Workshop on Stress in Yeast Cell Biology... and Beyond.**
Organizers: J. Ariño.

134 **Workshop on Leaf Development.**
Organizers: S. Hake and J. L. Micol.

---

*: Out of Stock.

The Centre for International Meetings on Biology
was created within the
Instituto Juan March de Estudios e Investigaciones,
a private foundation specialized in scientific activities
which complements the cultural work
of the *Fundación Juan March*.

The Centre endeavours to actively and
sistematically promote cooperation among Spanish
and foreign scientists working in the field of Biology,
through the organization of Workshops, Lecture
Courses, Seminars and Symposia.

From 1989 through 2001,
a total of 162 meetings,
all dealing with a wide range of
subjects of biological interest,
were organized within the
scope of the Centre.

Instituto Juan March de Estudios e Investigaciones
Castelló, 77 • 28006 Madrid (España)
Tel. 34 91 435 42 40 • Fax 34 91 576 34 20
http://www.march.es/biology

The lectures summarized in this publication were presented by their authors at a workshop held on the 25th through the 27th of February, 2002, at the Instituto Juan March.

All published articles are exact reproduction of author's text.

There is a limited edition of 450 copies of this volume, available free of charge.