UNIVERSIDAD DE ANTIOQUIA 1803

Revista Colombiana de Ciencias Pecuarias

http://rccp.udea.edu.co

RCCP

Contribution of the murine and primate models to the study of arenaviral diseases and hemorrhagic fevers ¤

Contribución de los modelos murino y primate al estudio de las enfermedades por arenavirus y fiebres hemorrágicas

Contribuição dos modelos murinos e primatas ao estudo de doenças por arenavirus e febre hemorrágica

Juan D Rodas[1], MV, MSc, PhD; Roger Hewson[2], PhD; María Salvato[3], PhD.

[1] *Grupo de Investigaciones en Ciencias Veterinarias "Centauro", Facultad de Ciencias Agrarias, Universidad de Antioquia, Medellín, Colombia AA1226,juandavid.rodas@gmail.com.*

[2] *Novel and Dangerous Pathogens, Virology, Centre for Emergency Preparedness and Response, Health Protection Agency – Porton Down, Salisbury, Wilts SP4 0JG, UK roger.hewson@hpa.org.uk.*

[3] *Institute of Human Virology; University of Maryland School of Medicine,725 West Lombard St., Baltimore, MD 21201, msalvato@ihv.umaryland.edu*

(Recibido: 2 junio, 2009 ; aceptado: 14 julio, 2009)

Summary

This manuscript is an inedited part of my PhD dissertation, based on historical and recent findings on animal models, that was presented as part of the requirements to fulfill the conditions to become a philosophical doctor on Veterinary Sciences at the University of Wisconsin on October of 2003.The current mini-review written on a free-version style, underlines some of the cornerstones of immunology as a science, understood thanks to the use of the Lymphocytic Choriomeningitis virus (LCMV) experimentally and naturally infected mouse model. It should suffice to say that there have been two Nobel prices of Medicine for discoveries made through the employment of this animal model, in order to recognize the right importance to it. In addition, several laboratories, Dr. Salvato´s among them, have also employed the LCMV-infected Rhesus monkey model as a tool to unravel the mysteries of arenaviral hemorrhagic fever, and particularly the physiopathology of Lassa disease in humans. Here I show some of the knowledge generated through the study of both animal infections.

Key words: *animal models, arenavirus, immunology, LCMV.*

¤ Para citar este artículo: Rodas JD, Hewson R, Salvato M. Contribution of the murine and primate models to the study of arenaviral diseases and hemorrhagic fevers. Rev Colomb Cienc Pecu 2009; 22: 267-277

* Autor para correspondencia: Facultad de Ciencias Agrarias, Universidad de Antioquia, Medellín, Colombia AA1226. E-mail: juandavid.rodas@gmail.com.

Resumen

El siguiente manuscrito, es un capítulo inédito de mi tesis doctoral, basado en hallazgos históricos y recientes sobre modelos animales, que fue presentado como parte de los requisitos para obtener el título de Ph.D. en Ciencias veterinarias en la Universidad de Wisconsin, en Octubre de 2003.La actual mini-revisión escrita en estilo de versión libre, subraya algunas de las piedras angulares de la inmunología como ciencia, entendidas gracias al uso del modelo murino infectado natural y experimentalmente con el virus de Coriomeningitis Linfocítica (LCMV). Sería suficiente mencionar que han existido dos premios Nóbel de Medicina por descubrimientos realizados a través del empleo de este modelo animal, para reconocer la real importancia del mismo. Adicionalmente, varios laboratorios, el de la Dra. Salvato entre ellos, también han empleado el modelo del mono Rhesus como un instrumento para desvelar los misterios de las fiebres hemorrágicas por arenavirus, y particularmente la fisiopatología de la enfermedad de Lassa en humanos. Aquí yo muestro alo del conocimiento generado a través del estudio de ambas infecciones animales.

Palabras clave: *arenavirus, inmunología, modelos animales, LCMV.*

Resumo

O seguinte manuscrito é um capítulo inédito da minha tese doutoral, baseado em casos históricos e recentes sobre modelos animais, que foram apresentados na defesa da tese de Ph.D. em Ciências Veterinárias da Universidade de Wisconsin, em outubro de 2003. A atual mini revisão escrita em estilo de versão livre, enfatiza em algumas pedras angulares da inmunologìa como ciência. Graças ao uso do modelo murino infectado natural e experimentalmente com o vírus da Coriomeningitis Linfocítica (LCMV). Seria suficiente mencionar que foram outorgados dois prêmios Nobel de Medicina pelos descobrimentos realizados a través do uso deste modelo, para reconhecer a real importância do mesmo. Adicionalmente, vários laboratórios, entre eles o da Dra. Salvato, tem utilizado o modelo macaco Rhesus como um instrumento para desvelar os mistérios das febres hemorrágicas por arenavirus, e particularmente a fisiologia e patologia da doença de Lassa em humanos. Aqui eu indico algo do conhecimento gerado a través do estudo das duas infecções animais.

Palavras chave: *arenavirus, inmunologia, modelos animais, LCMV.*

Introduction

The Arenaviridae are a family of enveloped viruses with bi-segmented, negative-stranded RNA genomes. Previous research has established arenaviruses as excellent probes of cell-mediated immunity in mice and as possible bio-threat agents in primates. Several laboratories have investigated the acute disease in primates by comparing virulent and avirulent strains of virus, with the goal of determining the molecular basis of virulence. Five years ago we practiced infections of rhesus macaques with the prototype arenavirus, lymphocytic choriomeningitis virus (LCMV) using the WE strain that has been known to cause both encephalopathy and multifocal hemorrhage, and serve to describe some of the fundamental aspects of the viral human hemorrhagic diseases. This discussion is inspired on some of those studies, but before that, is fair to give recognition to all the scientific data that made of LCMV in the mouse model, one of the best described and developed animal models for the study of both, the basic immune response and the understanding of the immune-mediated disease.

Contribution of the LCMV-infected mouse to immunology

Until the HIV era, the LCMV-infected mouse was the best-described model for cell-mediated immunity. This model was instrumental in the discovery of some of the most important concepts in immunology. In the 50's and 60's Rowe and Hotchin introduced the concept of immuno-pathogenesis, in other words the capacity of the immune system to cause disease (Hotchin, 1962; Rowe, 1954). Meanwhile, Rowe described how the immune system sometimes exerted only partial control of virus infection resulting in virus

persistence as a chronic infection (Rowe, 1954). In the 60's Hotchin and in the 80's Jacobson and Ahmed, showed that different isolates of LCMV had different tropisms and displayed various degrees of immuno-pathological disease (Ahmed *et al*, 1984; Hotchin, 1962; Jacobson and Pfau, 1980).

During the 70's Cole explained how cytotoxic T cells cause lethal pathology in the form of T cell-mediated choriomeningitis (Cole *et al*, 1972) but very soon Mims, Doherty and Zinkernagel, independently described how T cells also mediate anti-viral protection against LCMV (Doherty, 1976; Mims and Blanden, 1972; Zinkernagel, 1976). In 1974, again Zinkernagel and Doherty, using LCMV-infected cultures, described a fundamental tenet of cell-mediated immunity, MHC-restriction of the cytolytic reaction between effector T cells and their infected targets. They demonstrated how MHC class I regulates cytotoxic T cell immune response (Zinkernagel and Doherty, 1974), which brought them the Nobel prize in Medicine more than 20 years later (1996).

In 1977, Riviere described the detrimental effects of interferon on suckling mice and Welsh showed that natural killer cells, a source of interferon, were important effectors of innate immune responses after systemic virus infection (Riviere *et al*, 1977; Welsh Jr and. Zinkernagel, 1977). In the 80's Oldstone showed the feasibility of using cytotherapy with CD8+ cells to clear infected virus carriers (Oldstone *et al*, 1986). In the 90's Planz and coworkers treated infected carriers with $CD4^+$ T cells plus neutralizing antibody-producing B cells (Planz *et al*, 1997) based on older experiments from Volkert (1963). Also in1982, Oldstone and Buchmeier described the selective down-modulation of some viral transcripts during virus persistence *in vitro* and *in vivo* (Oldstone and Buchmeier, 1982).

Between 1968 and 1991, four labs (Leist *et al*, 1988; Mims and Wainwright, 1968; Odermatt *et al*, 1991; Silberman *et al*, 1978) described immunosupression by T cell-mediated anti-viral immunity; this mechanism also contributes to destruction of antigen presentation in AIDS (AlthageA *et al*, 1992; Borrow *et al*, 1995).

In the 1990's several experiments demonstrated the role of cytotoxic T cells in selecting virus, in causing immunological exhaustion or in evading immune detection. Pircher *et al* (1990) described the selection of LCMV mutants that escape CTL activity. In the next year Ohashi and Oldstone, independently, found that viral antigen on cells strictly outside of lymphoid tissue, such as pancreatic B-islet cells, are immunologically ignored. In contrast to infection with LCMV, immunization with a vaccinia-recombinant expressing the LCMV-Gp is not sufficient to cause disease, although it induces a potent, primed CTL response, thus suggesting that there is a high threshold for causing lymphocytic choriomeningitis (Ohashi *et al*, 1991; Oldstone *et al*, 1991).

In 1993, Moskophidis described exhaustion of precursor T cells by virus that spread throughout the lymphohemopoietic system, particularly LCMV-variants that replicate quickly and to high titers (Moskophidis *et al*, 1993). In 1994 Kagi and Walsh discover that perforin is essential for efficient, rapid $CD8^+$ T cell-dependent LCMV elimination (Kagi *et al*, 1994; Walsh *et al*, 1994). Four years later Klenerman described a case of "original antigenic sin" at the $CD8^+$ T cell level. This means that a CTL escape mutant virus may still induce a CTL response against the original wild-type virus that is not active against the mutant (Klenerman and Zinkernagel, 1998).

It has long been known that arenaviruses that persist in their murine host have been selected by humoral responses (Alche and Coto, 1988; Coto *et al*, 1981). In1999 Ochsenbein's research suggested that natural antibodies that are secreted and present in normal serum without immunization influence the initial virus distribution after LCMV infection (Ochsenbein *et al*, 1999). Finally in 2000 and 2001 Ciurea published that LCMV can persist by selection of mutant virus that escapes neutralizing antibody responses and $CD4^+$ T helper cell responses (Ciurea *et al*, 2001; Ciurea *et al*, 2000).

Still some issues remain unsolved in the mouse model for arenavirus research:

1. What is the reason behind the delay of neutralizing anti-LCMV responses? It is well known that neutralizing antibody responses against LCMV are delayed (Hotchin, 1962; Rowe, 1974). The delay suggests that viral antigen exhibiting the correct neutralizing epitope must still be available or even increased after day 50-100 at the time when neutralizing antibodies appear. CD8 elimination during the early phase of virus infection enhances generation of neutralizing antibody responses (Battegay *et al*, 1993; Planz *et al*, 1996). Both T cell-mediated general immuno-pathology and specific elimination of neutralizing antibody producing B cells may be involved.

2. What is the mechanism for survival of Cytotoxic T cell memory? While it is generally accepted that increased T (or B) cell frequencies of primed mice are antigen-independent, protective immunity against challenge with viruses (or tumors) outside of lymphoid organs is antigen-dependent (Bachmann *et al*, 1997; Kundig *et al*, 1996; Lau *et al*, 1994; Ochsenbein *et al*, 1999).

Could viral immuno-pathology described in the mouse contribute to primate hemorrhagic fever disease?

Immuno-pathology is a disease mediated by the immune response to a pathogen rather than by direct effect of the pathogen. Since many immune functions are controlled and restricted by host genetic factors, every individual responds uniquely and the outcome may vary greatly.

Immune-complexes that accumulate and deposit on organs such as kidney or spleen, are one source of immuno-pathology. Murine LCMV infection is considered a classical example of virus-induced immune complex disease. In this persistent LCMV infection non-neutralizing antibodies circulate in ratios and quantities that favor binding of complement and deposition in tissues that results in immunopathology. In a state that is affected by host and viral genetic factors (Oldstone *et al*, 1983), large quantities of immune-complexes along with IFN-γ contribute to glomerulo-nephritis (Walker and Murphy, 1987).

Innate immune responses represent another source of immuno-pathology. The induction of INF α/β by virus-infected cells occurs very rapidly (within a few hours of the infection) and represents an early and nonspecific antiviral response of the host. These molecules can inhibit the replication of many viruses through the induction of an "antiviral" state in the surrounding cells through the expression of: 1) 2'5' –oligoadenylate synthetases that mediates antiviral activities mainly by the induction of RNAse L, a cellular RNAse that degrades viral transcripts (Dong and Silverman, 1999; Terenzi *et al*, 1999), 2) the Mx proteins such as MxA protein (interferon-induced GTPase) that selectively inhibits influenza and bunyaviruses (Haller *et al*, 1998), 3) the double-stranded RNA-activated protein kinase (PKR), that apparently suppresses viral infections such as encephalomyocarditis virus and reovirus (Kalvakolanu and Borden, 1996).

In the LCMV-infected mouse model there are indications that IFN-α/β responses may promote disease under particular conditions. The first indication came from studies showing that the expression of these cytokines during LCMV infections of suckling mice is associated with the eventual inhibition of growth, liver necrosis, gromerulonephritis, and even death (Gresser, 1982; Riviere *et al*, 1980; Riviere *et al*, 1977). Through its role as regulator of viral spread, inducing the concentration of effectors cells in organs essential for life, type I IFNs could also contribute to the CD8 T cell-dependent death following intra-cranial infection with LCMV (Sandberg *et al*, 1994). A demonstration of this phenomenon was observed after blocking the functions of IFN-α/β with neutralizing antibodies, the virus disseminated widely and to a broader range of organs, protecting mice from the lethal consequences of the brain infection. In other words, the "dilution effect" of CD8 T cells from the brain to many other sites of viral infection saves life of the mouse.

Importantly, the early, virus-induced cytokine response may not be limited to INF α/β. Certain viruses such as LCMV and HIV have the potential to infect and activate macrophages, which secrete cytokines such as TNF-α (Guidotti *et al*, 1996) and Il-12 that induce IFN-γ (that also triggers other antiviral activities).

Another source of immuno-pathology is the response of antiviral lymphocytes, whose functions are not only to destroy infected cells expressing viral components presented in the MHC class I complex but also to secrete cytokines and chemokines that amplify the cellular immune response and have direct antiviral effects on infected cells (Von Herrath, 2002). Cytotoxic T lymphocytes effect some of the quickest and most specific antiviral immune response. There are at least 2 ways through which CD8 cells can be involved in immune-mediated viral diseases:

1. Cytolytically, they release perforins and induce the expression of Fas ligand that will result in apoptosis of virally infected cells (Kagi *et al*, 1994). A good example of immune-mediated pathogenesis where CD8+ cells are the key players, is the hepatitis induced in the mouse model after the ip inoculation with a viscerotropic strain of LCMV WE. Virus infection within the liver is predominantly confined to Kupffer cells from where it spreads to other cell types including hepatocytes. Apparently, the cellular damage is carried out by direct antiviral activity that is usually no cytotoxic.
   The number of infected hepatocytes is reduced, with less than 5% cellular destruction and this clearance is helped by the great regenerating capacity of the liver (Guidotti *et al*, 1996; Zinkernagel *et al*, 1986). In the HBV transgenic mouse model, there is an additional example of cell-mediated immune-pathology, where inefficient (or immature) CMI seems to lead to a serious condition known as cirrhosis.
   Cirrhosis is caused by progressive destruction and regeneration of hepatocytes, cirrhosis compromises the function of the organ and predisposes to liver cancer. In patients that develop cirrhosis cellular immune responses are restricted and weak in comparison to patients that overcome the acute HBV infection via high levels of IFN-γ and TNF-α that control the viral infection in a non- cytopathic way (Chisari, 1996).

2. Non-cytolitically, cytotoxic T cells secrete cytokines such as IFN-γ that not only increases antigen presentation by inducing expression of MHC class 1 and 2 but also induces TNF-α expression on macrophages (Guidotti and Chisari, 1996). IFN-γ also induces the release of NO (Guidotti *et al*, 2000), which affects viral targets such as the protease of Coxsackie virus (Saura *et al*, 1999; Zaragoza *et al*, 1997).
   TNF-α, in turn, increases expression of acute phase proteins such as IL-6 on Kupffer cells as part of the overall inflammatory response and proliferation after liver injury (Michalopoulos and DeFrances, 1997). More specifically, it has been shown that TNF-α also mediates down-regulation of hepatic HBV messengers (2.1-Kb mRNA) induced by IL-2 (Guilhot *et al*, 1993).
   A good example of cytokine-mediated disease is the mouse model for insulin-dependent diabetes mellitus (type I diabetes) where inflammatory cytokines such as IFN-γ, TNF-α and IL-1β are secreted by CTL and/or antiviral CD4 lymphocytes that mediate death of islet b cells. Apparently perforin-mediated killing of b cells by autoreactive CTL is not sufficient to produce clinically overt diabetes in vivo; a direct effect of IFN-γ produced by islet-infiltrating $CD4^+$ and $CD8^+$ lymphocytes is also required. Conversely, regulatory cells characterized by their ability to secrete cytokines such as IL-4, IL-10 and TGF-β can act in a suppressive regulatory manner if they home to a site targeted for autoimmune attack and prevent disease (Bach, 1994).

Very few examples have illustrated better the delicate balance between cell-mediated immunity and immune-mediated pathogenesis, than the transgenic HBV mouse (Ando *et al*, 1993) and the intra-cerebral LCMV infected mouse (Cole *et al*, 1971; Cole and Nathanson, 1974; Cole *et al*, 1972), repectivelly. In the first one the presence of CD8+ competent cells favor recovery, while in the second one the same cells induce lethal inflammation.

In a series of experiments with the hepatitis B virus (HBV) transgenic mouse, Chisari and Guidoti have demonstrated how IFN-γ and TNF-α secreted by activated T lymphocytes are the chief mechanisms for eliminating virus from liver in the absence of cell death (Guidotti *et al*, 1994; Guidotti and Chisari, 1999; Guidotti *et al*, 2002) . The rationale behind these studies is that viruses such as HBV outnumber the CTL by several orders of magnitude, yet the clearance in more than 95% of acutely infected adults could only be explained by the presence of cell mediators released by local macrophages and activated T cells. Viral clearance occurs in the absence of massive destruction or regeneration of hepatocytes and in the presence of inflammatory cells and Kupffer cell hyperplasia (Guidotti and Chisari, 1996). Their results suggest that HBV specific CTLs recognize and kill a small fraction of infected hepatocytes and later on, they secrete inflammatory cytokines that directly or indirectly (via macrophage activation) "cure" most of the infected hepatocytes by non-lytic intracellular inactivation pathways. Other authors have previously introduced the concept of cytokine-dependent "intracellular inactivation" for viruses such as herpes simplex (Martz and Howell *et al*, 1989).

A growing literature attributes further examples of cytokines controlling viral infections, and viruses encoding proteins that control cytokine activities, e.g. blocking the transcriptional activation of the IFN-activatable genes (Foster *et al*, 1991; Kalvakolanu *et al*, 1991), down-regulating TNF-α production *in vitro* (Adler *et al*, 1996) or presenting receptor analogues for IFN-α/β, IFN-γ and TNF-α (Alcami and Smith, 1995; McFadden *et al*, 1995; Guidotti and Chisari, 2000; Guidotti and Chisari, 2001; Schreiber and McFadden, 1994; Guidotti *et al*, 1999; Symons *et al*, 1995; Upton *et al*, 1992) , apparently as a strategy to blunt the antiviral activity of these cytokines.

In summary, if cytokines do play an effector role in the antiviral immune response, each virus can be expected to display its own cytokine sensitivity profile, and some viruses (including different subtypes of the same virus) may simply be insensitive to cytokine-mediated control. For example LCMV can establish a persistent infection *in vivo* in the presence of concentrations of TNFα and IFN-α/β that abolish HBV replication, thus LCMV is resistant to these cytokines in comparison with HBV (Guidotti and Chisari, 1996).

On the other hand, it has been suggested that the lethal choriomeningitis observed in the murine model intracerebraly inoculated with LCMV could be related to the deleterious effects of the inflammation caused by the presence of infiltrating cells. Even though the mechanism of death has not been completely elucidated, it is thought that besides the direct disruption of blood-brain barrier by lysis of infected cells and the increase of pressure in the skull produced by inflammatory cells, cytokines and other mediators of inflammation such as nitric oxide that are produced by infiltrating CD8+ T cells, could also be important effectors of the immune-mediated tissue damage (Borrow and Oldstone, 1997).

In conclusion, cytokines could work not only to abrogate viral replication but they are also potentially capable of inducing an immuno-pathological condition. In other words cytokines can help to prevent, cure or induce disease (Guidotti *et al*, 1996).

Evidence against immune-mediated pathology in the arenaviral hemorrhagic fever

Clinical studies of disease in the LCMV-infected mouse focused on the role of host-immune response to viral antigens as the actual cause of the disease. In contrast there is no evidence that the immune response exerts an adverse effect in the arenaviral hemorrhagic fever (HF). It has been shown, for instance, that immunosuppression with cyclosporin A or cyclophosphamide does not ameliorate disease of guinea pigs infected with the Junin virus. In fact these drugs affect the immune response to attenuated Junin viruses converting benign to lethal infections (Kenyon *et al*, 1985; Kenyon and Peters, 1986). The same results have been corroborated by others in different models: Machupo-infected monkeys (Eddy *et al*, 1975), Pichinde-infected guinea pigs (Johnson unpublished), Pichinde-infected hamsters (Murphy

et al, 1977), LCMV-infected guinea pigs (Buchmeier *et al*, unpublished) and LCMV-infected hamsters (Genovesi and Peters, 1987).

Another evidence such as the close correlation between the level of viral replication in vivo and the burden of clinical symptoms (Peters *et al*, 1987), also disputes the concept of immune-mediated pathogenesis by arenaviruses in different models of human HF. Even though in the mouse model, LCMV-WE can produce lethal immune-pathology of the liver (Bonilla *et al*, 2002) the disease differs from the one observed in primates in the low level of lymphocytic infiltration. In addition the major virulence determinants of LCMV WE (Riviere *et al*, 1985) and Lassa (Lukashevich, 1992; Lukashevich *et al*, 1991) are encoded on the large genomic segment and since this segment carries the genes that determine the level of virus replication, the amount of replication is likely to be the primary determinant of virulence.

Of course there are some other important factors that determine viral pathogenesis, for example the capacity to regulate mediators of inflammation such as IL-8. This capacity has been well characterized and mapped to the S segment of Lassa by comparing the in vitro induction levels of this cytokine after infection with the reassortant Lassa / Mopeia and the parental viruses (Lukashevich *et al*, 1999).

High circulating levels of IL-6, IL-6 receptor, and TNF receptors 1 and 2 are found in clinical cases of acute hepatitis in rhesus iv or ig inoculated with the WE strain of LCMV (Lukashevich *et al*, 2003) and they could be interpreted as signs of immune-mediated pathogenesis. However these factors seem to be more beneficial than detrimental since they help to regenerate injured hepatocytes (Michalopoulos and DeFrances, 1997). So it would be interesting to pursue additional and more conclusive experiments showing whether the inhibition of these specific cytokines or the depletion of other limbs of the immune response such as CD8, CD4, CD20 or even unspecific immune defense mechanisms such as complement, play a critical role in pathogenesis or immunity. Examples of these elegant approaches have been already performed on other models such as the chimpanzee infected with HBV or HCV (Thimme R, *et al.*, 2003) and SIV-infected rhesus monkeys (Schmitz *et al*, 1999; Schmitz *et al*, 2003; Schmitz *et al*, 1999).

Therefore, in spite of the negative experiments with immunosuppressive agents, the participation of the immune system in host damage has not been completely ruled out. Based on the studies from Chisari et al in the transgenic mouse model and the chimpanzee model for HBV it is plausible to speculate that we still do not know enough about the local and circulating level of apoptosis potentially induced by the population of CD8+ cells during the different stages of the LCMV-mediated hepatitis, nor do we have enough information about the role played by different important cytokines such as IFN-α, β and γ and TNF-α. As it has been described before, there is a precedent for the non-cythopatic viral clearance mediated by cytokines released by CD8+ cells activated during the acute infection with HBV.

Until the time comes when as we can manipulate the monkey model for hemorrhagic fever in such a way that we can explore the impact of different immunological effectors, as it has been done for the SIV- infected rhesus and the HBV-infected chimpanzee, we will rely on the indirect evidence that has already been presented. Future research should examine functional knock out of different arms of the immune system in order to determine the roles of immuno-pathology on hemorrhagic fever disease.

After having studied the LCMV-WE strain and its effects on the monkey model as a way to understand the pathogenesis of arenaviral hemorrhagic fevers, still some questions remain to be answered, among those are: What are the viral epitopes that elicit immune responses? A systematic study of Np and Gp peptides known from mouse studies, and using haplotyped monkey PBMC in immune-assays must be done in the future; the tools for doing CTL assays in the monkey could be greatly improved by identifying those. Interferon gamma secretion in response to peptides would also help to identify important CTL epitopes.

What is the nature and duration of mucosal immunity after the mucosal inoculations? To look at mucosal antibodies during the course of infection it would be necessary to collect fecal specimens for detection of soluble antibodies, and assay for the presence of virus specific Ig A. Other investigators have reported to biopsying mucosal sites to study the CTL and proliferative responses of mucosal lymphocytes (Rakasz *et al*, 2000). To do this work with so few effector cells, the peptide-based immune assays must first be developed. Mucosal infections could be confirmed by taking urine samples and looking for viral nucleic acids or titrating urine. In retrospect, that would support tremendously infection studies, since in some cases is hard to obtain evidence of infection from titration of blood. Future direction for mucosal studies would be very promising for the development of mucosal vaccines, an area that some labs have already explored with Salmonella / Lassa recombinants in mice (Djavani *et al*, 2001; Djavani *et al*, 2000). Future studies should elaborate a great many more details about the chronology of events leading to fatal hemorrhagic fever.

Finally, we need to reveal virus dissemination and gene expression changes that occur during the course of acute infection. In that sense and as an anecdotic comment, by the end of my academic training, I had the chance to participate in the necropsy of 10 monkeys that were inoculated with a lethal dose (10^3 PFU) of LCMV WE virus and euthanized each day for a week. The stored tissues were and have been studied to prove our hypothesis, what is that the liver is the main reservoir for virus replication, and that gene expression changes in the liver contribute to the multi-organ disaster that is hemorrhagic fever. Some of the results of these studies have been published during the course of the last three years after my departure from my advisor´s lab, and some more are still to come and are already accepted for publication in the Memorias of the Institute Oswaldo Cruz from Brazil.

In summary, unlike the aberrant disease immune response-mediated to the artificial intracranial inoculation in the mouse model, the response to a viral infection in which the disease is proportional to dose in the monkey model, must be closely examined in the infected organs and use to learn the sequence of events leading to death and to determine the possible targets for therapeutic interventions or best suited response to prevent fatal outcomes.

Acknowledgments

Many thanks to my mentor Maria Salvato, for her ideas and constructive discussions, that are the inspiration of this article.

References

Adler H, Jungi TW, Pfister H, Strasser M, Sileghem M *et al*. Cytokine regulation by virus infection: bovine viral diarrhea virus, a flavivirus, downregulates production of tumor necrosis factor alpha in macrophages in vitro. J Virol 1996; 70:2650-3.

Ahmed R, Salmi A, Butler LD, Chiller JM, and Oldstone MB. Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice. Role in suppression of cytotoxic T lymphocyte response and viral persistence. J Exp Med 1984; 160:521-40.

Alcami A, and Smith GL. Vaccinia, cowpox, and camelpox viruses encode soluble gamma interferon receptors with novel broad species specificity. J Virol 1995; 69:4633-9.

Alche LE, and Coto CE. Differentiation of Junin virus and antigenic variants isolated in vivo by kinetic neutralization assays. J Gen Virol 1988; 69 (Pt 8):2123-7.

AlthageA, Odermatt B, Moskophidis D, Kundig T, Hoffman-Rohrer U *et al*. Immunosuppression by lymphocytic choriomeningitis virus infection: competent effector T and B cells but impaired antigen presentation. Eur J Immunol 1992; 22:1803-12.

Ando K, Moriyama T, Guidotti LG, Wirth S, Schreiber RD *et al*. Mechanisms of class I restricted immunopathology. A transgenic mouse model of fulminant hepatitis. J Exp Med 1993; 178:1541-54.

Bach JF. Insulin-dependent *diabetes mellitus* as an autoimmune disease. Endocr Rev 1994; 15:516-42.

Bachmann MF, Kundig TM, Hengartner H and Zinkernagel RM. Protection against immunopathological consequences of a viral infection by activated but not resting cytotoxic T cells: T cell memory without „memory T cells“? Proc Natl Acad Sci U S A 1997; 94:640-5.

Battegay M, Kyburz D, Hengartner H and Zinkernagel RM. Enhancement of disease by neutralizing antiviral antibodies in the absence of primed antiviral cytotoxic T cells. Eur J Immunol 1993; 23:3236-41.

Bonilla WV, Pinschewer DD, Klenerman P, Rousson V, Gaboli M *et al*. Effects of promyelocytic leukemia protein on virus-host balance. J Virol 2002; 76:3810-8.

Borrow P, Evans CF and Oldstone MB. Virus-induced immunosuppression: immune system-mediated destruction of virus-infected dendritic cells results in generalized immune suppression. J Virol 1995; 69:1059-70.

Borrow P and Oldstone MBA. Lymphocytic Chroriomeningitis Virus, p. 593-627. *In* N. N. e. al. (ed.), Viral pathogenesis, First ed. Lippincott-Raven, Philadelphia. 1997.

Chisari FV. Hepatitis B virus transgenic mice: models of viral immunobiology and pathogenesis. Curr Top Microbiol Immunol 1996; 206:149-73.

Ciurea A, Hunziker L, Martinic MM, Oxenius A, Hengartner H *et al*. CD4+ T-cell-epitope escape mutant virus selected in vivo. Nat Med 2001; 7:795-800.

Ciurea A, Klenerman P, Hunziker L, Horvath E, Senn BM *et al*. Viral persistence in vivo through selection of neutralizing antibody-escape variants. Proc Natl Acad Sci U S A 2000; 97:2749-54.

Cole GA, Gilden DH, Monjan AA and Nathanson N. Lymphocytic choriomeningitis virus: pathogenesis of acute central nervous system disease. Fed Proc 1971; 30:1831-41.

Cole GA and Nathanson N. Lymphocytic choriomeningitis. Pathogenesis. Prog Med Virol 1974; 18:94-110.

Cole GA, Nathanson N and Prendergast RA. Requirement for theta-bearing cells in lymphocytic choriomeningitis virus-induced central nervous system disease. Nature 1972; 238:335-7.

Coto C, Vidal MC, D'Aiutolo AC and Damonte E. Selection of spontaneous ts mutants of Junin and Tacaribe viruses in persistent infections, p. 59-64. *In* B. D.H.L. and C. R.W. (ed.). The replication of negative strand viruses. Elsevier-North Holland, New York. 1981.

Djavani M, Yin C, Lukashevich IS, Rodas J, Rai SK *et al*. Mucosal immunization with *Salmonella typhimurium* expressing Lassa virus nucleocapsid protein cross-protects mice from lethal challenge with lymphocytic choriomeningitis virus. J Hum Virol 2001; 4:103-8.

Djavani M, Yin C, Xia L, Lukashevich IS, Pauza CD *et al*. Murine immune responses to mucosally delivered Salmonella expressing Lassa fever virus nucleoprotein. Vaccine 2000; 18:1543-54.

Doherty PC, Dunlop MB, Parish CR and Zinkernagel RM. Inflammatory process in murine lymphocytic choriomeningitis is maximal in H-2K or H-2D compatible interactions. J Immunol 1976; 117:187-90.

Dong B, and Silverman RH. Alternative function of a protein kinase homology domain in 2', 5'-oligoadenylate dependent RNase L. Nucleic Acids Res 1999; 27:439-45.

Eddy GA, Scott SK, Wagner FS and Brand OM. Pathogenesis of Machupo virus infection in primates. Bull World Health Organ 1975; 52:517-21.

Foster GR, Ackrill AM, Goldin RD, Kerr IM, Thomas HC *et al*. Stark. Expression of the terminal protein region of hepatitis B virus inhibits cellular responses to interferons alpha and gamma and double-stranded RNA. Proc Natl Acad Sci U S A 1991; 88:2888-92.

Genovesi EV and Peters CJ. Immunosuppression-induced susceptibility of inbred hamsters (Mesocricetus auratus) to lethal-disease by lymphocytic choriomeningitis virus infection. Arch Virol 1987; 97:61-76.

Gresser, I. Can interferon induce disease? Interferon 1982; 4:95-127.

Guidotti LG, Ando K, Hobbs MV, Ishikawa T, Runkel L *et al*. Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a noncytolytic mechanism in transgenic mice. Proc Natl Acad Sci U S A 1994; 91:3764-8.

Guidotti LG, Borrow P, Hobbs MV, Matzke B, Gresser I *et al*. Viral cross talk: intracellular inactivation of the hepatitis B virus during an unrelated viral infection of the liver. Proc Natl Acad Sci U S A 1996; 93:4589-94.

Guidotti LG and Chisari FV. Cytokine-induced viral purging--role in viral pathogenesis. Curr Opin Microbiol 1999; 2:388-91.

Guidotti LG and Chisari FV. Cytokine-mediated control of viral infections. Virology 2000; 273:221-7.

Guidotti LG and Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. Annu Rev Immunol 2001; 19:65-91.

Guidotti LG and Chisari FV. To kill or to cure: options in host defense against viral infection. Curr Opin Immunol 1996; 8:478-83.

Guidotti LG, McClary H, Loudis JM and Chisari FV. Nitric oxide inhibits hepatitis B virus replication in the livers of transgenic mice. J Exp Med 2000; 191:1247-52.

Guidotti LG, Morris A, Mendez H, Koch R, Silverman RH *et al*. Interferon-regulated pathways that control hepatitis B virus replication in transgenic mice. J Virol 2002; 76:2617-21.

Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R et al. Viral clearance without destruction of infected cells during acute HBV infection. Science 1999; 284:825-9.

Guilhot S, Guidotti LG and Chisari FV. Interleukin-2 downregulates hepatitis B virus gene expression in transgenic mice by a posttranscriptional mechanism. J Virol 1993, 67:7444-9.

Haller O, Frese M and Kochs G. Mx proteins: mediators of innate resistance to RNA viruses. Rev Sci Tech 1998; 17:220-30.

Hotchin J. The biology of lymphocytic choriomeningitis infection: virus induced immune disease. Cold Spring Harb Symp Quant Biol:479-499. 1962.

Jacobson S and Pfau CJ. Viral pathogenesis and resistance to defective interfering particles. Nature 1980; 283:311-3.

Kagi D, Ledermann B, Burki K, Seiler P, Odermatt B *et al*. Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. Nature 1994; 369:31-7.

Kagi D, Vignaux F, Ledermann B, Burki K, Depraetere V *et al*. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. Science 1994; 265:528-30.

Kalvakolanu DV, Bandyopadhyay SK, Harter ML and Sen GC. Inhibition of interferon-inducible gene expression by adenovirus E1A proteins: block in transcriptional complex formation. Proc Natl Acad Sci U S A 1991; 88:7459-63.

Kalvakolanu DV and Borden EC. An overview of the interferon system: signal transduction and mechanisms of action. Cancer Invest 1996; 14:25-53.

Kenyon RH, Green DE and Peters CJ. Effect of immunosuppression on experimental Argentine hemorrhagic fever in guinea pigs. J Virol 1985; 53:75-80.

Kenyon RH and Peters CJ. Cytolysis of Junin infected target cells by immune guinea pig spleen cells. Microb Pathog 1986; 1:453-64.

Klenerman P and Zinkernagel RM. Original antigenic sin impairs cytotoxic T lymphocyte responses to viruses bearing variant epitopes. Nature 1998; 394:482-5.

Kundig TM., Bachmann MF, Oehen S, Hoffmann UW, Simard JJ *et al*. On the role of antigen in maintaining cytotoxic T-cell memory. Proc Natl Acad Sci U S A 1996; 93:9716-23.

Lau LL, Jamieson BD, Somasundaram T and Ahmed R. Cytotoxic T-cell memory without antigen. Nature 1994; 369:648-52.

Leist TP, Ruedi E and Zinkernagel RM. Virus-triggered immune suppression in mice caused by virus-specific cytotoxic T cells. J Exp Med 1988; 167:1749-54.

Lukashevich IS. Generation of reassortants between African arenaviruses. Virology 1992; 188:600-5.

Lukashevich IS, Maryankova R, Vladyko AS, Nashkevich N, Koleda S *et al*. Lassa and Mopeia virus replication in human monocytes/macrophages and in endothelial cells: different effects on IL-8 and TNF-alpha gene expression. J Med Virol 1999; 59:552-60.

Lukashevich IS, Tikhonov I, Rodas JD, Zapata JC, Yang Y *et al*. Arenavirus-mediated liver pathology: acute lymphocytic choriomeningitis virus infection of rhesus macaques is characterized by high-level interleukin-6 expression and hepatocyte proliferation. J Virol 2003; 77:1727-37.

Lukashevich IS, Vasiuchkov AD, Stel'makh TA, Scheslenok EP and Shabanov AG. [The isolation and characteristics of reassortants between the Lassa and Mopeia arenaviruses]. Vopr Virusol 1991; 36:146-50.

Martz E and Howell DM. CTL: virus control cells first and cytolytic cells second? DNA fragmentation, apoptosis and the prelytic halt hypothesis. Immunol Today 1989; 10:79-86.

McFadden G, Graham K, Ellison K, Barry M, Macen J *et al*. Interruption of cytokine networks by poxviruses: lessons from myxoma virus. J Leukoc Biol 1995; 57:731-8.

Michalopoulos GK and DeFrances MC. Liver regeneration. Science 1997; 276:60-6.

Mims CA and Blanden RV. Antiviral action of immune lymphocytes in mice infected with lymphocytic choriomeningitis virus. Infect Immun 1972; 6:695-8.

Mims CA and Wainwright S. The immunodepressive action of lymphocytic choriomeningitis virus in mice. J Immunol 1968; 101:717-24.

Moskophidis D, Lechner F, Pircher H and Zinkernagel RM. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature 1993; 362:758-61.

Murphy FA, Buchmeier MJ and Rawls WE. The reticuloendothelium as the target in a virus infection. Pichinde virus pathogenesis in two strains of hamsters. Lab Invest 1977; 37:502-15.

Ochsenbein AF, Fehr T, Lutz C, Suter M, Brombacher F et al. Control of early viral and bacterial distribution and disease by natural antibodies. Science 1999; 286:2156-9.

Ochsenbein AF, Karrer U, Klenerman P, Althage A, Ciurea A *et al*. A comparison of T cell memory against the same antigen induced by virus versus intracellular bacteria. Proc Natl Acad Sci U S A 1999; 96:9293-8.

Odermatt B, Eppler M, Leist TP, Hengartner H and Zinkernagel RM. Virus-triggered acquired immunodeficiency by cytotoxic T-cell-dependent destruction of antigen-presenting cells and lymph follicle structure. Proc Natl Acad Sci U S A 1991; 88:8252-6.

Ohashi PS, Oehen S, Buerki K, Pircher H, Ohashi CT *et al*. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. Cell 1991; 65:305-17.

Oldstone MB, Blount P, Southern PJ and Lampert PW. Cytoimmunotherapy for persistent virus infection reveals a unique clearance pattern from the central nervous system. Nature 1986; 321:239-43.

Oldstone MB and Buchmeier MJ. Restricted expression of viral glycoprotein in cells of persistently infected mice. Nature 1982; 300:360-2.

Oldstone MB, Nerenberg M, Southern P, Price J and Lewicki H. Virus infection triggers insulin-dependent *diabetes mellitus* in a transgenic model: role of anti-self (virus) immune response. Cell 1991; 65:319-31.

Oldstone MB, Tishon A and Buchmeier MJ. Virus-induced immune complex disease: genetic control of C1q binding complexes in the circulation of mice persistently infected with lymphocytic choriomeningitis virus. J Immunol 1983; 130:912-8.

Peters CJ, Jahrling PB, Liu CT, Kenyon RH, McKee KT *et al*. Experimental studies of arenaviral hemorrhagic fevers. Curr Top Microbiol Immunol 1987; 134:5-68.

Pircher H, Moskophidis D, Rohrer U, Burki K, Hengartner H *et al*. Viral escape by selection of cytotoxic T cell-resistant virus variants in vivo. Nature 1990; 346:629-33.

Planz O, Ehl S, Furrer E, Horvath E, Brundler MA et al. A critical role for neutralizing-antibody-producing B cells, CD4(+) T cells, and interferons in persistent and acute infections of mice with lymphocytic choriomeningitis virus: implications for adoptive immunotherapy of virus carriers. Proc Natl Acad Sci U S A 1997; 94:6874-9.

Planz O, Seiler P, Hengartner H and Zinkernagel RM. Specific cytotoxic T cells eliminate B cells producing virus-neutralizing antibodies [corrected]. Nature 1996; 382:726-9.

Rakasz E, MacDougall AV, Zayas MT, Helgelund JL, Ruckward TJ *et al*. Gammadelta T cell receptor repertoire in blood and colonic mucosa of rhesus macaques. J Med Primatol 2000; 29:387-96.

Riviere Y, Ahmed R, Southern PJ, Buchmeier MJ and Oldstone MB. Genetic mapping of lymphocytic choriomeningitis virus pathogenicity: virulence in guinea pigs is associated with the L RNA segment. J Virol 1985; 55:704-9.

Riviere Y, Gresser I, Guillon JC, Bandu MT, Ronco P *et al*. Severity of lymphocytic choriomeningitis virus disease in different strains of suckling mice correlates with increasing amounts of endogenous interferon. J Exp Med 1980; 152:633-40.

Riviere Y, Gresser I, Guillon JC and Tovey MG. Inhibition by anti-interferon serum of lymphocytic choriomeningitis virus disease in suckling mice. Proc Natl Acad Sci U S A 1977; 74:2135-9.

Rowe WP. Studies on pahtogenesis and immunity in lymphocytic choriomeningitis infection of the mouse. Navy Research Report: 167-220. 1954.

Sandberg K, Kemper P, Stalder A, Zhang J, Hobbs MV *et al*. Altered tissue distribution of viral replication and T cell spreading is pivotal in the protection against fatal lymphocytic choriomeningitis in mice after neutralization of IFN-alpha/beta. J Immunol 1994; 153:220-31.

Saura M., Zaragoza C, McMillan A, Quick RA, Hohenadl C *et al*. An antiviral mechanism of nitric oxide: inhibition of a viral protease. Immunity 1999; 10:21-8.

Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA *et al*. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 1999; 283:857-60.

Schmitz JE, Kuroda MJ, Santra S, Simon MA, Lifton MA *et al*. Effect of humoral immune responses on controlling viremia during primary infection of rhesus monkeys with simian immunodeficiency virus. J Virol 2003; 77:2165-73.

Schmitz JE, Lifton MA, Reimann KA, Montefiori DC, Shen L *et al*. Effect of complement consumption by cobra venom factor on the the course of primary infection with simian immunodeficiency virus in rhesus monkeys. AIDS Res Hum Retroviruses 1999; 15:195-202.

Schreiber M and McFadden G. The myxoma virus TNF-receptor homologue (T2) inhibits tumor necrosis factor-alpha in a species-specific fashion. Virology 1994; 204:692-705.

Silberman SL, Jacobs RP and Cole GA. Mechanisms of hemopoietic and immunological dysfunction induced by lymphocytic choriomeningitis virus. Infect Immun 1978; 19:533-9.

Symons JA, Alcami A and Smith GL. Vaccinia virus encodes a soluble type I interferon receptor of novel structure and broad species specificity. Cell 1995; 81:551-60.

Terenzi F, deVeer MJ, Ying H, Restifo NP, Williams BR *et al*. The antiviral enzymes PKR and RNase L suppress gene expression from viral and non-viral based vectors. Nucleic Acids Res 1999; 27:4369-75.

Thimme R, Wieland S, Steiger C, Ghrayeb J, Reimann KA et al. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol 2003; 77:68-76.

Upton C, Mossman K and McFadden G. Encoding of a homolog of the IFN-gamma receptor by myxoma virus. Science 1992; 258:1369-72.

Volkert M. Studies on immunological tolerance to LCMV. 2. Treatment of virus carrier mice by adoptive immunization. Acta Pathol Microbiol Scand 1963; 57: 465–87.

von Herrath MG. Regulation of virally induced autoimmunity and immunopathology: contribution of LCMV transgenic models to understanding autoimmune insulin-dependent diabetes mellitus. Curr Top Microbiol Immunol 2002; 263:145-75.

Walker DH and Murphy FA. Pathology and pathogenesis of arenavirus infections. Curr Top Microbiol Immunol 1987; 133:89-113.

Walsh CM, Matloubian M, Liu CC, Ueda R, Kurahara CG *et al*. Immune function in mice lacking the perforin gene. Proc Natl Acad Sci U S A 1994; 91:10854-8.

Welsh RM Jr and. Zinkernagel RM. Heterospecific cytotoxic cell activity induced during the first three days of acute lymphocytic choriomeningitis virus infection in mice. Nature 1977; 268:646-8.

Zaragoza C, Ocampo CJ, Saura M, McMillan A and Lowenstein CJ. Nitric oxide inhibition of coxsackievirus replication in vitro. J Clin Invest 1997; 100:1760-7.

Zinkernagel RM and Doherty PC. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. Nature 1974; 248:701-2.

Zinkernagel RM, Dunlop MB, Blanden RV, Doherty PC and Shreffler DC. H-2 compatibility requirement for virus-specific T-cell-mediated cytolysis. Evaluation of the role of H-2I region and non-H-2 genes in regulating immune response. J Exp Med 1976; 144:519-32.

Zinkernagel RM, Haenseler E, Leist T, Cerny A, Hengartner H *et al*. T cell-mediated hepatitis in mice infected with lymphocytic choriomeningitis virus. Liver cell destruction by H-2 class I-restricted virus-specific cytotoxic T cells as a physiological correlate of the 51Cr-release assay? J Exp Med 1986; 164:1075-92.