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Message from the Director General

At Institut Pasteur of Shanghai we have a clear goal: to contribute to a better public health in China and in the world through excellence in research, through sharing of our knowledge and translation of our science to concrete public health tools.

The institute is now strong of about 240 staff including 200 researchers and students. We will further grow to up to 400 staff within the next 3 years. In 2010 we have increased our research output and signed further partnership agreements. In 2012, IPS will move to its new 16,000m² building on the SIBS campus in the Xuhui district. As we are growing in size we will enhance our research focus. Research in Infectious Diseases and related domains like immunology and virus-induced cancer will remain our main focus areas. Translational research and early stage preclinical and clinical proof of concept studies will be driven by centers dedicated to specific areas like vaccine, diagnostic or therapeutic R&D and the development of the bio-incubator subsidiary of IPS, Advance BioChina.

China's current and future economic growth will transform the healthcare sector. At IPS we are in an excellent strategic position to make significant contributions to this growing healthcare sector through our excellence in science and our dedication to contribute to public health in China.

We look forward to the opportunities to work with partners worldwide to achieve our goals.



Ralf Altmeyer
Director General



Bing Sun
Co-Director

2010 highlights

In 2010, the scientific output of IPS-CAS is increasing fast. 20 papers published in *Journal of Virology*, *Journal of Immunology*, *Journal of Biological Chemistry*, *PLoS One*, *Retrovirology*, *Virology*, *Journal of Clinical Virology*. The grant collection has also significantly improved, a sum of 18M RMB was awarded.

IPS-CAS was approved to create ‘CAS Key Lab on Molecular Virology and Immunology’. IPS-CAS aims to apply for accreditation of state key laboratory in field of virology and immunology in the coming years.

Three new teams were established at IPS in 2010. The principal investigators are Prof. Ralf Altmeier, (‘Anti-Infective Research’); Prof. Hui Xiao, (‘Immune Signaling and Regulation’), and Prof. Dongming Zhou, (‘Anti-infection immunity and vaccine research’).

The establishment of Pathogen Diagnosis Center (PDC) has improved IPS-CAS capacity as a platform for translational research and emergence response. The PDC is playing an active role in the regional surveillance network of Pasteur Institutes. The PDC was designated by Shanghai CDC as one of 6 external support laboratories during Shanghai Expo

IPS-CAS is fulfilling its strong commitment to education and training. In 2010, IPS-CAS was proud to witness its first 3 students with Ph.D degree. They are: Cheguo Cai, Shijian Zhang and Wei Wang.

IPS-CAS officially started its global biotech incubator/accelerator “Advance BioChina”, a proactive, broad business development initiative towards global and local actors of the biotech and pharmaceutical industry, to accelerate and facilitate their China market access

Research

Research Strategy

- Basic research on severe infectious diseases (Virology and Immunology)
- New diagnosis approach
- Novel vaccine

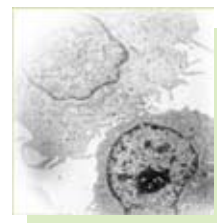
Focus on diseases that impact China

Scientists at IPS unravel mechanisms of disease and develop novel approaches to diagnosis, vaccination and therapeutic intervention



Hand, Foot and Mouth Diseases is on the rise in China. HFMD affected more than 3 million children since 2008 and caused over 1,200 deaths due to severe neurological and cardiovascular complications.

HIV/AIDS and related tumors are the leading cause of death from infectious diseases in China. A vaccine is urgently needed.



Acute Respiratory Infections (Influenza, Para influenza, etc) cause significant morbidity and mortality worldwide. Viruses from five different families cause ARI and require monitoring, novel therapies and vaccines to avoid future epidemics and pandemics.

Viral hepatitis caused by Hepatitis B and C viruses accounts for 140 million chronic carries in China and leads to liver cirrhosis and liver cancer in millions of patients.



List of research units

Unit	Principal Investigator	Year of establishment
Molecular Virology	B. SUN	2005
Antiviral Immunity and Genetic Therapy	P. ZHOU	2005
Viral Genome Regulation	T.TOYODA	2005
Tumor Virology	K. LAN	2006
Viral Hepatitis	J.ZHONG	2006
Immune Regulation	Q.B LENG	2007
Structural Virology	R.CHEN	2007
Viral Immunology	JH WANG	2009
Vaccinology and Antiviral Strategies	Zh.HUANG	2009
Molecular Immunology	B. LI	2009
Hematopoietic Stem Cell and Transgenic Animal Model	Y.ZHANG	2009
Innate Immunity	GX Meng	2009
Immune Signaling and Regulation	H. Xiao	2010
Anti-infection immunity and vaccine research	D.M.Zhou	2010
Anti-Infective Research	R.Altmeyer	2010
Dendritic Cell Biology and Viral Sensor	Y.J. Liu(Visiting Professor)	2009
Viral Immunology and Vaccines	R.F.Wang(Visiting Professor)	2009

Unit of Molecular Virology (Established in 2005)

Principal Investigator

Bing Sun

Ph.D in Immunology from Shanghai Second Medical University;

Postdoctoral Fellow (1994-1996) and Visiting Scientist (1996-1999) at NIH (USA)

Team members

Assistant Principle Investigator: Ke Xu, Ph.D.

M.S-Ph.D students: Chen Xu, Kai Wang, Yuan He, Leilei Yang, Weibin Hu, Shiqi Xie, Qinglin Han

Intern: Ronghua Zhang, Yan Wang, Hong Zhang

Technicians: Wenjing Yu, Yan Zeng, Tongyan Wang, Chong Chang

Administrative staff: Wenjing Xuan

Research objective

We are dedicated in understanding the molecular mechanisms for the pathogenesis in virus infection and trying to identify host factors that restrict virus infection in human. Human respiratory viruses (influenza A viruses) and hepatitis C virus (HCV) were selected to study their pathogenesis in virus infection and host factors that control virus infection. Human coronaviruses (SARS, OC43 and 229E) and Enterovirus 71 (which cause Hand foot mouth disease) were selected to study 3a-like and 2B proteins serving as viral ion channel proteins in regulating virus release. We mainly focus on the ion channel properties of certain viral proteins and viral-host interactions as well as viral-host cross talk through the innate immunity.

Highlights of achievements and progress

1. Study on viral ion-channel proteins

Besides our first identification of viral ion-channel protein in SARS-CoV (*Proc Natl Acad Sci*, 2006, 103:12540-12545.), we further find that many human coronaviruses including HCoV-229E of Class I, HCoV-OC43 of Class II and SARS-CoV of Class IIb all encode homology viroporins, ORF3a, which function as an ion channels. In swine coronavirus, we also found that ORF3 in PEDV (Porcine Epidemic Diarrhea Virus) presents an ion channel property, and may contribute to virus release (paper in preparation).

More importantly, our viroporin research has extend to the newly epidemic human virus EV71(Enterovirus 71) circulating in children in Asia-pacific region. We find that EV71 2B protein induces a chloride current when it is expressed in *Xenopus* oocytes. A chloride channel

inhibitor, DIDS, reduces virus-induced cytopathic effect and virus particles release in RD cells. These data suggest that 2B protein may play an important role in the virus production and is a potential anti-viral drug target (Fig.1, Cell Research, in Revision).

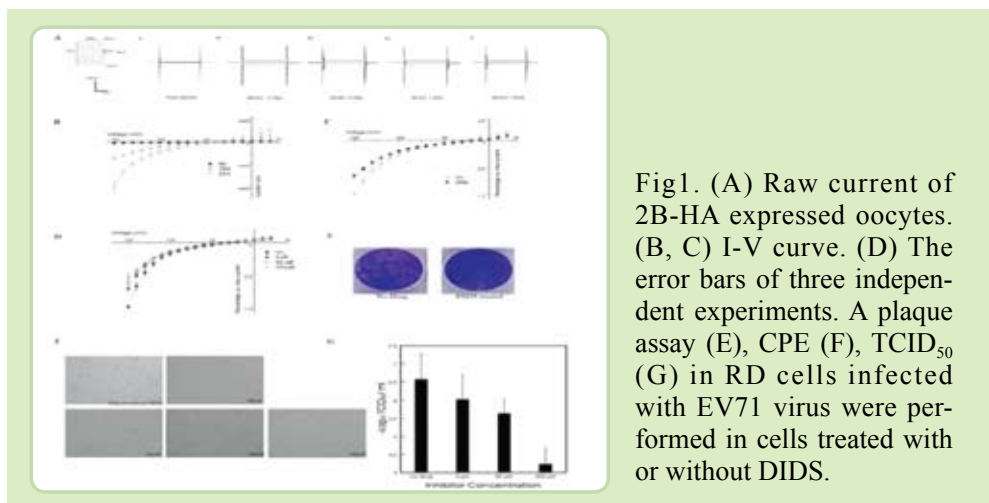


Fig1. (A) Raw current of 2B-HA expressed oocytes. (B, C) I-V curve. (D) The error bars of three independent experiments. A plaque assay (E), CPE (F), TCID₅₀ (G) in RD cells infected with EV71 virus were performed in cells treated with or without DIDS.

2. Interplay of influenza A virus and the host systems

Influenza A virus is highly variable and a major viral respiratory pathogen that can cause severe illness in human. The non-structural protein 1 (NS1 protein) and polymerase complex are two major factors for efficient infection rate and high virulence of influenza A virus.

We find that NS1 of a highly pathogenic avian influenza H5N1 virus interacts with human Ubc9 and is SUMOylated in transfected and infected cells at its C-terminus. SUMOylation enhances NS1 stability and thus promotes rapid growth of influenza A virus. Studies on different influenza A virus strains of human and avian origins showed that the majority of viruses possess a NS1 protein that is modified by SUMO1, except for the recently emerged swine-origin influenza A virus (S-OIV) H1N1. (Fig.2, *Journal of Virology*, 2011, Jan. 85(2):1086–98)

Many viruses interact with the host cell division cycle to favor their own growth. We find that influenza A virus replication results in G0/G1 phase accumulation of infected cells to facilitate viral protein expression and progeny virus production. The G0/G1 phase cell cycle arrest is likely to be a common strategy since the effect was also observed in other strains such as H3N2, H9N2, PR8 H1N1 and pandemic swine H1N1 viruses (Fig.3, *Journal of Virology*, 2010, Dec; 84(24):12832-40).

A lysine (K) residue in PB2 627 results in high RNA polymerase activity and viral replication of influenza A virus in mammalian cells. When 627K is mutated to glutamine (E), polymerase activity is reduced and viral replication is restricted. We demonstrate that the PB1 subunit (especially residues 473V and 598P) of a H5N1 PB2-627E virus plays a key role in maintaining polymerase activity in human cells (In Prepare).

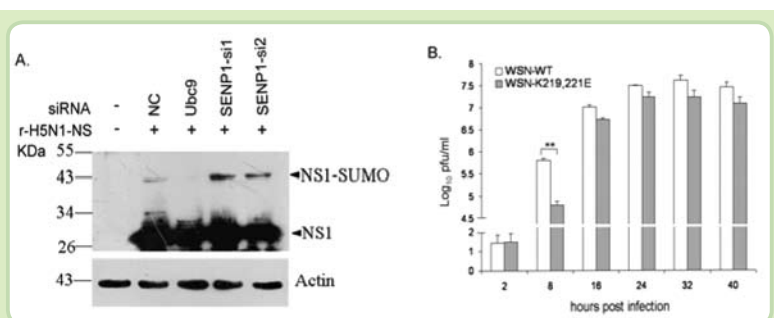


Fig2. (A) SUMOylation of influenza A virus NS1 protein in infected A549 cells. (B) SUMOylation of NS1 in WT virus promotes rapid virus growth in early infection stage.

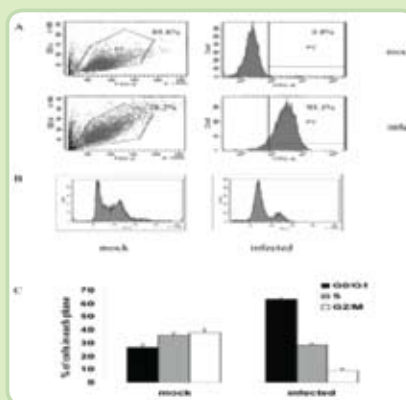


Fig3. G0/G1 phase accumulation induced by influenza A virus A/WSN/33 (H1N1) infection

3. Development of efficient influenza vaccines

We have developed an efficient DNA vaccine against influenza A virus, which expresses both HA and NP genes in a bi-promotor plasmid. This DNA vaccine is able to elicit both efficient humoral and cellular immune responses to homo- and hetero- subtypic viruses (*Viral Immunol*, 2011 Feb; 24(1):45-56).

Moreover, we find that K119N mutation in HA1 protein is responsible for the improved virus growth of NIBRG-121xp, an egg-adaptive vaccine strain for 2009 swine-origin H1N1 influenza virus (S-OIV), in eggs with intact antigenicity (In preparation).

4. Host factors in counteraction with HCV infection

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease world widely. Based on cDNA microarray data (36,000 genes), protein-motif or domain analysis and available bioinformatics analysis, we have identified a new host factor, tripartite motif-containing 22 (TRIM22) stimulated about 15.7-fold by IFN α and up-regulated during HCV infection in Huh-7 cells. Over-expression of TRIM22 suppressed HCV replication.

Grants

- NSFC “3a and its homologous proteins regulate coronaviruses release mechanism and durg target study” (2009.1-2011.12);
- MOST: “The pathogenicity of RNA polymerase and NS1 suppression on IFN- α/β production in different strains of avian flu viruses” (2009.1-2012.1);
- NSFC: “The pathogenesis to the drug design and surveillane the variance of H1N1 flu” (2009.6-2010.5);
- CAS “The new adjuvant gp96 and the variance research of H1N1 flu” (2009.6-2010.5).

Collaboration (national and international)

- Participate in MOST project (the pathogenesis of influenza virus A infection)
- H. D. Klenk in Institut fuer Virologie, Marburg, Germany (AIV study);
- Yuelong Shu in Beijing CDC (AIV study);
- Jinhua LIU in China Agriculture University (AIV study);
- Ze Chen and Tianxian LI Wuhan Institute of Virology, CAS (AIV study);
- Wolfgang Schwarz, Max-Planck-Institut, (ion-channel research);
- Bojian, Zheng in Hong Kong University (SARS study);
- Shanghai Institute of Pharmaceutical Industry (anti-virus drug screening).
- Hualan Biological Engineering Inc. (Vaccine research)

Presentations at invitation at the national and international conferences

Bing Sun

14th International Congress of Immunology, Kobe, Japan, Aug., 2010
“Inflammatory cytokines and anti-microbial responses”

Bing Sun

The 4th Chinese-Karolinska Institute Medical Symposium, Stockholm ,Sweden, Oct., 2010
“Viral respiratory diseases, such as SARS and avian influenza”

Bing Sun

Future of sciences, sciences for the future: Chemistry and its interfaces with biology and physics, Paris, France, Oct., 2010
“Viral respiratory diseases, such as SARS and avian influenza”

Bing Sun

CSI-IFReC Joint Symposium on Immunology, Hangzhou, China, Nov 2010
TRIM30 negatively regulates TLR-mediated NF- κ B activation

Perspectives

- Set up well-standard ion-channel platform and extend to study other interesting viral ion channel proteins. Drug screen against these viral ion channels.
- Set up well-established Influenza Virus polymerase activity study system and reverse genetics system to understanding the remaining questions on influenza research.
- Set up cell cycle analysis and SUMOylation analysis method on viral proteins, explore the similar function in other interesting viral proteins.
- Understand the effects of innate immunity molecular on HCV life cycle.
- Explore new interactions between host and virus in highly concerned viruses such as influenza virus and HCV.

Publications

Xu K, Klenk C, Liu B, Keiner B, Cheng J, Zheng BJ, Li L, Han Q, Wang C, Li T, Chen Z, Shu Y, Liu J, Klenk HD, Sun B*. SUMO1 modification of the non-structural protein 1 of influenza A virus. *Journal of Virology*. 2011 Jan;85(2):1086-98.

He Y, Xu K, Keiner B, Zhou J, Czudai V, Li T, Chen Z, Liu J, Klenk HD, Shu YL, Sun B*. Influenza A virus replication Induces Cell Cycle Arrest in G0/G1 Phase. *Journal of Virology*. 2010 Dec; 84(24):12832-40.

Wang K, Xie S, Sun B*. Viral proteins function as ion channels. *Biochim Biophys Acta*. 2011 Feb;1808(2):510-5. Epub 2010 May 15.

Xu K, Ling ZY, Sun L, Xu Y, Bian C, He Y, Lu W, Chen Z, Sun B*. A broad humoral and cellular immunity elicited by a bivalent DNA vaccine encoding HA and NP genes from a H5N1 virus. *Viral Immunol*. 2011 Feb;24(1):45-56.



Unit of antiviral immunity and genetic therapy (Established in 2005)

Principal Investigator

Paul Zhou

Ph.D in Immunology from School of Medicine, State University of New York at Buffalo, USA;
Postdoctoral Fellow in Immunology from Mayo Clinic, USA (1989-1993);
Senior Staff Fellow, Oral Infection and Immunity Branch, NIH (1993-1998);
Principal Investigator at Department of Virology and Immunology of Southwest Foundation for Biomedical Research and Adjunct Associate Professor at Department of Microbiology of University of Texas, USA (1998-2005).

Team members

M.S.-Ph.D students: Hongxing Hu, Heng Ding, Lifei Yang, Yufeng Song, Weiming Wang, Fan Zhou, Lihong Liu

Graduated students with Ph.D degree: Cheguo Tsai (2010), Michael Wen (2010)

Senior research assistant: Guiqin Wang

Research assistant: Lulan Wang, Jingjing Liu, Shumei Wang, Wensi Yi

Secretary: Qingzhi Zhang

Research objectives

1. Membrane-bound antibody-based strategy for therapy and prevention against HIV

Since the failure of the T cell-based phase IIb clinical vaccine trial (STEP) in 2007, HIV-1 vaccine field focuses more on broad neutralizing antibodies (their generation, induction and mechanism of action, etc.). Previously we showed that a non-neutralizing human anti-HIV-1 gp41 antibody (TG15) can be turned into a broad neutralizing antibody when expressed on the surface of HIV-1 susceptible cells. We named this approach as membrane-bound antibodies (Lee et al. *J. Immunol.* 2004). For the past years we have developed a new form of membrane-bound antibodies by genetically linking several single chain Fvs (scFvs; AB65, TG15, AB31, AB32, 4E10, X5 and 48d) with a glycosylphosphatidylinositol (GPI)-attachment signal anchor. We demonstrated that 1) with a GPI-attachment signal scFvs are targeted into lipid raft of cytoplasm membrane; 2) GPI-scFvs (TG15, AB32, 4E10, X5 and 48d) exhibit various degrees of breadth and potency against diverse HIV-1 strains and among them GPI-scFv (X5) remarkably

inhibits all diverse HIV-1 strains tested with a great degree of potency. Thus, we conclude that GPI-anchored scFv is an effective way to capture transiently exposed neutralization epitopes on HIV-1 spike and the GPI-scFv (X5) with such broad and potent neutralization activity should have great potential to be developed into anti-HIV-1 agents in prevention and in therapy. A manuscript based on these findings was published (Wen et al. *Retrovirology* 7:79, 2010).

Currently we are focusing on two aspects of GPI-scFv approach. First, we are systematically testing the effect of GPI-scFv (X5) on transmission of HIV-1 captured by dendritic cells (DC) to CD4⁺ cells. We demonstrated that GPI-scFv (X5) effectively blocks both *cis*- and *trans*-transfers of HIV-1 from immature DC to CD4⁺ cells as well as *trans*-transfer of HIV-1 from mature DC to CD4⁺ cells. Second, in collaboration with Dr. Ping Zhong at the Shanghai CDC, we are constructing lentiviral vectors expressing GPI-scFv library from memory B cells of HIV-1 infected individuals whose plasmas exhibit broad neutralization activity against diverse HIV-1 strains. GPI-scFv library will then be transduced and displayed on the surface of HIV-1-susceptible cells. Meanwhile, we are making recombinant HIV-1 expressing unique reporter genes that will allow us to screen in and out individual GPI-scFvs that block HIV-1 entry. We believe that once established, this novel screen system will not only allow us to identify scFvs that recognize novel neutralization epitopes (constitutively or transiently exposed) on HIV-1 envelope spike, but also is applicable to identify neutralization epitopes on envelope spikes of many other viruses.

2. Influenza HA and NA pseudotype-based neutralization assay and its utilities

Neutralizing antibody responses are critical for virus prevention and clearance. Microneutralization (MN) and the hemagglutination inhibition (HI) assays are two conventional assays used to evaluate neutralizing antibody responses against highly pathogenic avian influenza (HPAI) H5N1 viruses. However, due to the use of replication competent HPAI viruses both assays require biosafety level 3 (BSL-3) containment facilities. Therefore, a neutralization assay that does not require BSL-3 facilities would be advantageous. Previously, we generated a panel of influenza HA and NA pseudotypes. Using the pseudotypes we developed a HA/NA pseudotype-based neutralization (PN) assay. In collaboration with Dr. Fred Vogel at Sanofi Pasteur we demonstrated that the PN assay is a sensitive and quantifiable assay in measuring neutralizing antibodies against diverse H5N1 viruses (Tsai et al. *Vaccine* 2009). Currently the PN assay has been used in various studies on vaccine and human monoclonal antibody development against influenza viruses.

First, we have extended our HA and NA pseudotype panel including all clades and subclades of H5 HA. Meanwhile, we have generated a panel of mouse immune sera against individual clades and subclades of H5 HA. Using these pseudotype and immune serum panels, we have performed across-board neutralization assays. By so doing, we have determined cross reactivity of diverse clades and subclades of H5 HA. Based on the cross reactivity data, we are designing

and testing immunogens that will elicit broad neutralizing antibody responses against all clades and subclades of H5N1 viruses.

Second, we have studied on heterosubtypic neutralizing antibody response between the seasonal influenza vaccine and potential pandemic influenza viruses. Using a sensitive PN assay we tested heterosubtypic neutralizing antibody responses to H5N1 viruses elicited with seasonal influenza vaccines in humans and in mice. We demonstrated that low levels of heterosubtypic neutralizing antibody response against H5N1 virus were indeed elicited with seasonal influenza vaccine in humans and in mice. Importantly, we showed that 2 of 27 mice whose immune sera exhibited similar levels of neutralizing antibody response as homosubtypic H5N1 VLP actually survived from HPAI H5N1 virus challenge, indicating at certain levels such heterosubtypic neutralizing antibody response offers immune protection against severity of H5N1 virus infection (Ding et al. **PLoS ONE** in press).

Third, we used the assay in evaluation of vaccine candidates. Although DNA plasmid and virus-like particle (VLP) vaccines have been individually tested against HPAI H5N1 viruses, the combination of both vaccines into a heterologous prime-boost strategy against HPAI H5N1 viruses has not been reported before. Therefore, we constructed DNA plasmid encoding H5HA and generated VLP expressing the same H5HA and N1NA. We then compared neutralizing antibody responses and immune protection elicited with heterologous DNA-VLP, homologous DNA-DNA and VLP-VLP prime-boost strategies in mice. We demonstrate that superior neutralizing antibody response and protection in mice vaccinated with heterologous DNA prime and VLP boost against HPAI H5N1 virus. Thus, our results provide strong support for clinical evaluation of heterologous DNA-VLP prime-boost strategy as a public health intervention against a possible H5N1 pandemic (Ding et al. **PLoS ONE** 6(1):e16563, 2011).

Finally, in collaboration with John Skehel at National Institute for Medical Research, Mill Hill, London, United Kingdom and Boping Zhou at the Shenzhen Third Hospital we used the PN assay to screen human monoclonal antibodies produced by immortalized memory B cells from a H5N1 infected individual. By molecular cloning techniques, we developed three fully human monoclonal antibodies. Among them, one such antibody 65C6 exhibited potent neutralization activity against all clades of H5N1 viruses and prophylactic and therapeutic efficacy against highly pathogenic avian influenza H5N1 viruses in mice. Studies on HA-antibody complexes by electron microscopy indicate that antibody 65C6 binds to an epitope on the tip of the membrane-distal globular domain of HA. A patent application based on these human monoclonal antibodies is being written and will be filed soon. A manuscript based on these findings has been written and will be submitted soon. Currently, we are defining amino acid residues evolving in the neutralization epitope as well as the mechanism of neutralization by antibody 65C6.

3. Virus like particle (VLP) immunogens against HIV and influenza A viruses

Vaccination is a cost-effective means to combat viral infection. Historically, live attenuated

viruses have been used as vaccine candidates to control many viral infections. However, some live attenuated virus vaccines such as HIV have been limited to animal studies, but not human clinical trials due to the safety concerns. In an attempt to structurally mimic virus particle, but without potential risks involving some live attenuated viruses, virus like particles (VLP) have been developed and used as immunogens. There are several advantages of using VLP as immunogens. First, since the VLP does not replicate and does not contain viral genome, they avoid the formidable safety concerns associated with whole-inactivated and live attenuated viral vaccines. Second, the immune system responds well to particulate antigens that are the size of viruses. Third, envelope proteins on the surface of VLP are likely in their native forms to elicit desirable antibody responses. Finally, by recombinant technology many new features can be incorporated into VLP to improve their immunogenicity.

However, the current systems to produce VLP of enveloped viruses such as HIV and influenza viruses have many limitations. Therefore, to overcome these limitations in VLP production, we have developed a novel system to produce VLP (Here the details of the system are purposely omitted for the reason of IP). So far, we demonstrated that using this system HIV-1 envelope and gag proteins are properly expressed, released and incorporated into VLP. HIV-1 envelope proteins are properly processed and glycosylated mainly in high mannose form. Preliminary quantification indicates that the amount of HIV-1 envelope proteins at least comparable, if not higher, to those produced by Sf9 cells transduced by recombinant baculovirus. In addition, we also demonstrated that DNA prime and VLP (with or without adjuvants) boost elicit both CD4 and CD8 T cell responses. Both HIV-1 envelope and gag (to lesser extent) peptide-specific CD8 T cells were detected. DNA prime and VLP (with or without adjuvants) boost elicit good ELISA-binding and neutralizing antibody responses against HIV-1 envelope proteins. A patent application based on this novel VLP production system has recently filed.

4. Human –simian immunodeficiency virus (HSIV) chimera-infected pigtailed macaque model

The species-specific tropism of HIV-1, the primary cause of AIDS, has hindered the development of an animal model of infection and disease. Among nonhuman primates commonly used for AIDS research, the pig-tailed macaque is uniquely susceptible to HIV-1, although infection does not persist or cause disease due to an intrinsic block to viral replication. In collaboration with Drs. Jason Kimata at the Baylor College of Medicine in Houston and Shiu-Lok Hu at the Washington National Primate Center in Seattle, USA, we first showed much higher transduction efficiency of VSV-G-pseudotyped HIV-1 vector in PBMCs isolated from pig-tailed macaques than rhesus macaques. We then demonstrated that robust replication of HIV-1 in pig-tail T cells after substituting the viral infectivity factor protein, *vif*, with that from SIV. Finally, we showed that the HIV-1(N-L4-3)-SIV-*vif* chimera was persistent in two adult pigtailed macaques for 12 months post infection. A manuscript based on these findings was accepted (Thippeshappa et al. *J. Virol.* in press). Currently, additional HSIV chimeras have been constructed and tested in

human and pigtailed macaque cell lines. The results looked quite promising. In a few weeks, Weiming Wang and I (Paul Zhou) will visit Dr. Shiu-Lok Hu's laboratory the Washington National Primate Center to test these new chimeras in pigtailed macaque PBMCs. If these chimeras have good *in vitro* growth kinetics in pigtailed macaque PBMCs, they will be inoculated into pigtailed macaques to test their replication, growth and immunogenicity *in vivo*. I strongly believe that this HSIV-infected pigtailed macaque model, if succeeded, will have an enormous impact in HIV vaccine development. Here, I'd like to thank the Shanghai Pasteur Health Foundation and the Areva for their grant money that supports this study.

Collaborators

- Boping Zhou Donghu Infectious Diseases Hospital, Shenzhen, China
- John Skehel National Institutes of Medical Research, MRC, Mill Hill, UK
- Vincent Deubel Institute Pasteur of Cambodia
- Jason Kimata Barloy College of Medicine, Houston, USA
- Shui-Lok Hu Washington National Primate Center, Seattle, USA

Grants currently funded

- Project Leader in the grant funded by the Li Kai-Shing Foundation entitled "Optimization of influenza HA-based pseudotypes and their use in development of a novel neutralizing assay" (2006.7 to 2011.6)
- Principal Investigator in the Shanghai Pasteur Foundation program grant entitled "Develop humanized mouse model for evaluating efficacy and safety of vaccine candidates against HIV" (2008.1 to 2011.12)
- Project Leader in the Grand Science and Technology Special Project on Vaccine against HIV 2008ZX1001-1010-03 entitled "Research on VLP" (2008.1 to 2011.3)
- Project Leader in the Grand Science and Technology Special Project on Platform Development for Diagnosis of Respiratory and CNS Viral Infection 2009ZX10004-105-05 entitled "Pseudotype-based Assay for Measuring Neutralizing Antibody Responses against HPAI H5N1 viruses" (2008.1 to 2011.6)
- Principal Investigator in the Areva program grant entitled "Develop membrane-bound antibodies against HIV" (2009.1 to 2011.12)

Papers published and accepted in the last 12 months

Original research articles

Wen, M., Arora, R., Wang, H., Liu, L., Kimata, J.T., and **Zhou, P.** GPI-anchored single chain Fv - an effective 1 way to capture transiently-exposed neutralization epitopes on HIV-1 envelope

spike. *Retrovirology* 7:79-90, 2010

Ding, H., Tsai, C., Gutiérrez, R. A., Zhou, F., Buchy, P., Deubel, V., and **Zhou, P.** Superior Neutralizing Antibody Response and Protection in Mice Vaccinated with Heterologous DNA Prime and Virus Like Particle Boost against HPAI H5N1 Virus. *PLoS ONE* 6(1):e16563, 2011

Li, R., Qin Y., He, Y., Tao, W.Y., Zhang, N., Tsai, C.G., **Zhou, P.**, and Zhong, J. Production of hepatitis C virus lacking the envelope-encoding genes for single-cycle infection by providing homologous envelope proteins or vesicular stomatitis virus glycoproteins in trans. *J. Virol.* in press

Thippeshappa, R., Polacino, P., Kimata, M.Y., Siwak, E.B., Anderson, D., Wang, W., Sherwood, L., Arora, R., Wen, M., **Zhou, P.**, Hu, S-L., and Kimata, J.T. Vif Substitution Enables Persistent Infection of Pig-tailed 1 Macaques By Human Immunodeficiency virus type. *J. Virol.* in press

Ding, H., Tsai, C., Gutiérrez, R. A., Zhou, F., Buchy, P., Deubel, V., and **Zhou, P.** Heterosubtypic Antibody Response Elicited with Seasonal Influenza Vaccine Correlates Partial Protection against Highly Pathogenic H5N1 Virus. *PLoS ONE* in press

Review

Mascola, J., King, C.R., Steinman, R., Alter, G., Burton, D.R., Karlsson-Hedestam, G., Le Grand, R., Liljestrom, P., Liu, M., Liu, Y-J., McElrath, J., Nikolic, B., Nussenzweig, M., Panicali, D., Pensiero, M., Sallusto, F., Seder, R., Stamatatos, L., Williamson, A-L., Yewdell, J., and **Zhou, P.** Immunogens and Antigen Processing: Report from a Global HIV Vaccine Enterprise Working Group. *Nature Medicine* 2010 doi:10.1038/npre.2010.4796.2

Presentations by the unit members at national and international conferences

Michael Wen

China AIDS Vaccine Symposium, Shanghai, China, May, 2010

“GPI-anchored antibodies: an effective way to capture transiently exposed neutralization epitopes on HIV-1 spike”

Yufong Song

China AIDS Vaccine Symposium, Shanghai, China, May, 2010

“Update in VLP immunogen development against HIV/AIDS”

Paul Zhou

4th IPS-Areva Course, Shanghai, China, May, 2010

“Human neutralizing antibodies against HIV-1 and influenza viruses”

Cheguo Tsai

VII Influenza Conference, Hong Kong, September, 2010

“Heterosubtypic Antibody Response Elicited with Seasonal Influenza Vaccine Correlates Partial Protection against Highly Pathogenic H5N1 Virus”

Heng Ding

VII Influenza Conference, Hong Kong, September, 2010

“Superior Neutralizing Antibody Response and Protection in Mice Vaccinated with Heterologous DNA Prime and Virus Like Particle Boost against HPAI H5N1 Virus”

Hongxing Hu

VII Influenza conference, Hong Kong, September, 2010

“Broad neutralizing human monoclonal antibodies against H5N1 viruses”

Michael Wen

10th AIDS Vaccine Conference, Atlanta, USA, October, 2010

“GPI-anchored antibodies: an effective way to capture transiently exposed neutralization epitopes on HIV-1 spike”



Tumor Virology Unit (Established in 2006)

Principal Investigator

Ke LAN

MD., Ph.D. in Pathophysiology, Central South University, China (2001).

Postdoctoral fellow at Department of Microbiology, University of Pennsylvania, USA (2002-2006).

Team member

Co-Principal investigator: Qiang Deng, Associate Professor

Research Assistant, Haitao Mao

Technician: Xin Yu

Postdoctoral fellows: Yuan Gao, Xing Wang, Min Wei

Ph.D. students: Tian Xia, Zhiheng He, Yunhua Liu, Deguang Liang, Xiaofan Li, Xianzhi Lin, Yi Jin, Zhuo Wang, Baosen Jia, Zhihua Qi, Qinglan Zhao

MS-Ph.D. students: Rui Sun, Lei Bai, Quanzhi Zhang

Research objective

Tumor Virology Unit is dedicated to elucidating the mechanisms by which tumor viruses mediated oncogenesis. Kaposi's Sarcoma Associated Herpesvirus (KSHV) and Hepatitis B virus (HBV) are utilised the working models to address the mechanisms of viral persistence, tissue injury (e.g., tumorigenesis, angiogenesis or immune damage), and antiviral responses. Basically, we are focusing on two major topics of, 1) the mechanisms of viral latency control as well as related pathogenesis of KHSV; 2) development of mouse models of HBV chronic infection and the potential anti-viral strategy based on active immunotherapy.

Highlights of achievements and progress

1. miR-K12-11 attenuates interferon signaling and contributes to maintenance of KSHV latency by targeting IKK ϵ

Type I interferon (IFN) signaling is the principal response mediating antiviral innate immunity. IFN transcription is dependent upon the activation of transcription factors IRF3/IRF7 and NF- κ B. Numerous viral proteins have been shown as being capable of interfering with IFN signaling to facilitate evasion from the host innate immune response. However, it is not determined that whether viral miRNAs are also involved in IFN signaling regulation.

By bioinformation analysis, IKK ϵ (I-kappa-B kinase epsilon) was predicted as a potential target of the miR-K12-11 encoded by KSHV. IKK ϵ is a non-canonical IKK (I-kappa B kinase) related kinase, which is responsible for IRF3 and IRF7 activation in multiple IFN signaling pathways. We found that miR-K12-11 repressed IKK ϵ 3' UTR reporter activity in a dose dependent manner. Moreover, we found that ectopic expression of miR-K12-11 resulted in decreased IKK ϵ expression, while inhibition of miR-K12-11 was found to restore IKK ϵ expression in KSHV infected cells. Importantly, expression of miR-K12-11 attenuated IFN signaling by decreasing IKK ϵ -mediated IRF3/IRF7 phosphorylation and by inhibiting the activation of IKK ϵ -dependant IFN Stimulating Genes (ISGs), allowing miR-K12-11 suppression of antiviral immunity. Thus, our data suggest that IKK ϵ targeting by miR-K12-11 is an important strategy utilized by KSHV to modulate IFN signaling.

We also demonstrated that IKK ϵ was able to enhance KSHV reactivation synergistically with the treatment of 12-*O*-tetradecanoylphorbol 13-acetate (TPA). Moreover, inhibition of miR-K12-11 enhanced KSHV reactivation induced by vesicular stomatitis virus (VSV) infection. Taken together, our findings also suggest that miR-K12-11 can contribute to maintenance of KSHV latency by targeting of IKK ϵ . This work was published in **Cell Research**.

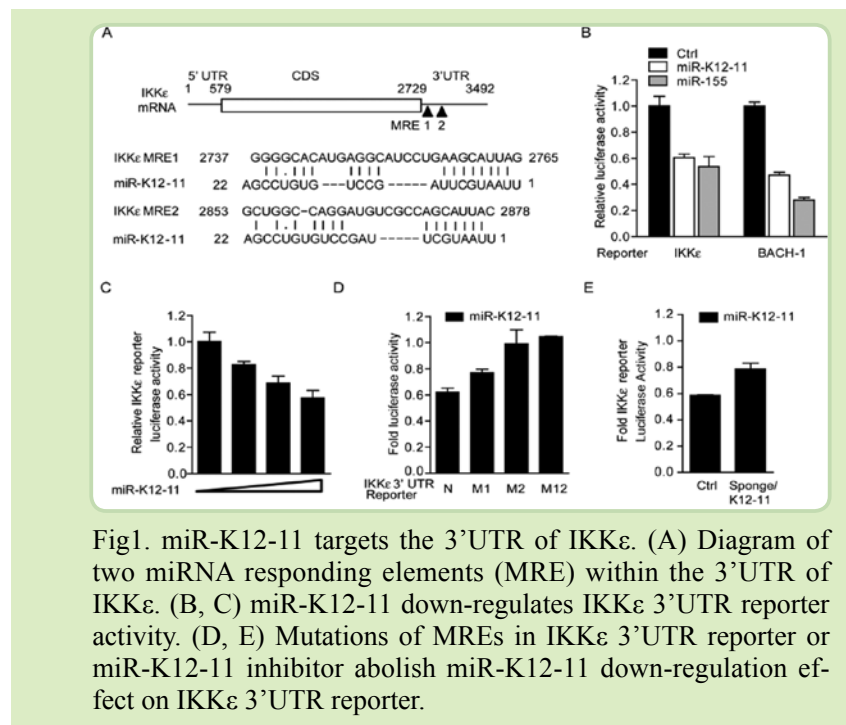


Fig1. miR-K12-11 targets the 3'UTR of IKK ϵ . (A) Diagram of two miRNA responding elements (MRE) within the 3'UTR of IKK ϵ . (B, C) miR-K12-11 down-regulates IKK ϵ 3'UTR reporter activity. (D, E) Mutations of MREs in IKK ϵ 3'UTR reporter or miR-K12-11 inhibitor abolish miR-K12-11 down-regulation effect on IKK ϵ 3'UTR reporter.

2. miR-K12-7-5p encoded by Kaposi's Sarcoma-Associated Herpesvirus stabilizes the latent state by targeting viral ORF50/RTA

KSHV has been reported to express 17 mature miRNAs from 12 pre-miRNAs during viral latency. Through a different strategy from earlier researches, we screened these pre-miRNA expression plasmids using a luciferase reporter construct containing the 3' untranslated region (3'UTR) of the RTA gene (RTA3'UTR). Among these miRNAs, miR-K12-7, together with miR-K12-9, inhibited the expression of RTA, the major regulator of the viral life cycle. miR-K12-7 targeted RTA3'UTR in a seed sequence-dependent manner. miR-K12-7-5p derived from miR-K12-7 mediates the inhibition of RTA expression, and the mutation of the seed match site totally abrogated the inhibitory effect of miR-K12-7 on RTA3'UTR. The inhibition of RTA expression by miR-K12-7 was further confirmed in the latently KSHV-infected 293/Bac36 cell line through transient transfection of miR-K12-7 expression plasmid or specific inhibitor of miR-K12-7-5p, respectively. The transient transfection of miR-K12-7 into 293/Bac36 cells reduced RTA expression and the expression of the downstream early genes regulated by RTA, and also the production of progeny virus was significantly reduced after treatment with chemical inducers. Taken together, this research revealed that another miRNA, miR-K12-7-5p, targets the viral immediate early gene RTA and that this miRNA contributes to the maintenance of viral latency. This work was published in **Plos One**.

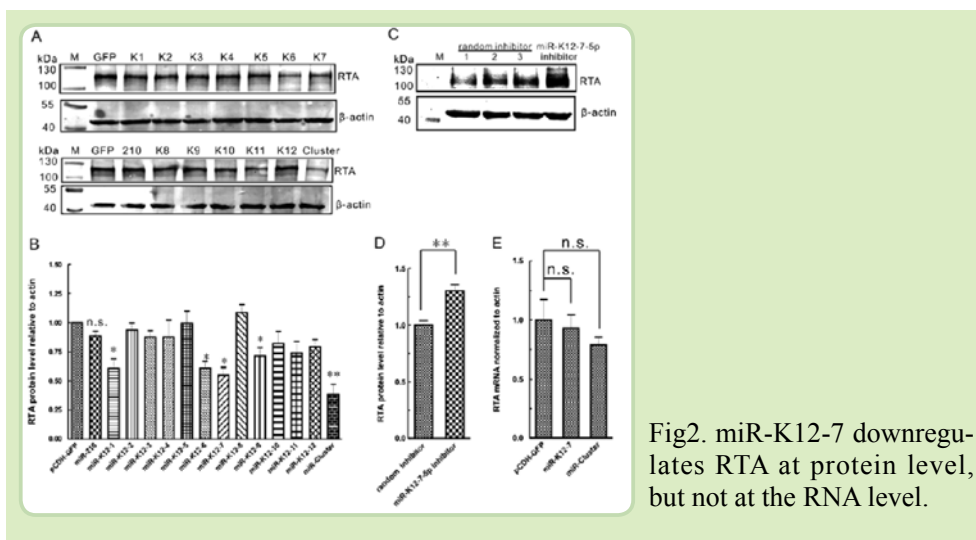
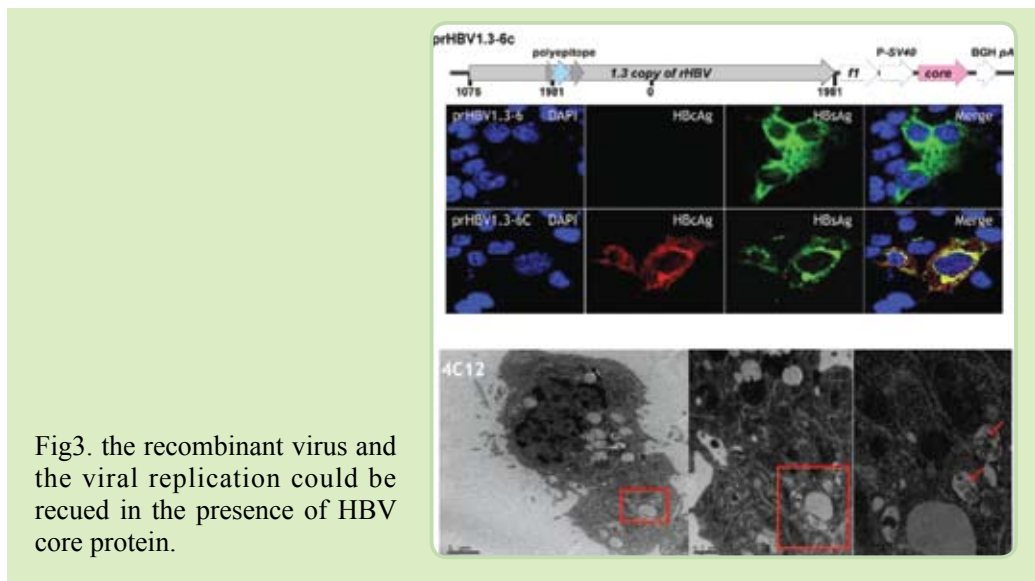


Fig2. miR-K12-7 downregulates RTA at protein level, but not at the RNA level.

3. A novel strategy to overcome the potential immune tolerance during HBV persistent infection

Hepatitis B virus (HBV) represents a major cause of human disease worldwide. To set up an active immunotherapy, immune response should be switched from exhausted T cells to func-

tional effectors that are able to reach liver and cure the viral infection. To this goal, we designed a recombinant HBV (rHBV) with modified viral *core* gene that carried a foreign polypeptide coding sequence within HBV genome. The recombinant virus could self-maintain only in HBV-hosting hepatocytes through capsid complementation. Experiments performed in HLA-A2/HBsAg transgenic mice further validated our approach. As shown in Fig 3, rHBV replication could be rescued by the complementation of the core protein *in trans* (upper panel). The 42 nm rHBV virions were visualized intracellularly under the electronic microscope (bottom panel). The recombinant viral particles proved to be infectious to cultured HepaRG cells *in vitro*. For therapeutic delivery, the rHBV genome was further subcloned to an adenovirus vector system which elicited a strong foreign antigen specific T cell response in HLA-A2/DR1 mice. The ongoing study will be nailed down soon.



Grants

- Principal Investigator, Natural Science Foundation of China grant entitled “Mechanism of KSHV latency control” (2008.1 to 2011.12)
- Principal Investigator, Shanghai Pasteur Foundation grant entitled “Interplay between KSHV and HIV” (2008.1 to 2011.12)
- Principal Investigator, CAS 100 Talent program grant entitled “KSHV infection & related oncogenesis” (2008.8 to 2011.8)
- Principal Investigator, TOTAL Program grant entitled “HBV related oncogenesis” (2008.7 to 2011.7)

- Principal Investigator, CAS Knowledge Innovation Program grant entitled “Vaccine study for HIV & hepatitis viruses” (2007.8 to 2011.8)
- Principal Investigator, 973 MOST grant entitled “Basic studies for HCV infection control” (2009.1 to 2013.9)
- Principal Investigator, National Eleventh Five-year Plan Science & Technology Key project (Development of platform for detection of respiratory & CNS viruses)(2009.1 to 2011.6)
- Principal Investigator, National Eleventh Five-year Plan Science & Technology Key project (Epidemiology study of virus infection in Shanghai population)(2009.1 to 2011.4)
- Principal Investigator, Natural Science Foundation of China grant entitled “Study on the interaction between TLE2 and KSHV RTA” (2010.1 to 2013.12)
- Principal Investigator, 973 MOST grant entitled “Molecular mechanism of KSHV latent infection” (2011.1 to 2015.12)

Collaboration (national and international)

- Dr. Erle S. Robertson (University of Pennsylvania) – For KSHV latency study.
- Dr. Yan Yuan (University of Pennsylvania) – For KSHV reactivation study.
- Dr. Fanxiu Zhu (Florida State University) – For KSHV reactivation study.
- Dr. Hao Wen (Xinjiang Medical University) – For KSHV epidemiology study.
- Dr. Yu Wei (Institut Pasteur) – For HBV viral entry study.
- Dr. Dazhi Zhang (Chongqing Medical University) – For HBV viral entry study.
- Dr. Marie Louise Michel (Institut Pasteur of Paris) – For HBV vaccination study.

Perspective

Overall, Tumor Virology Unit was running smoothly and steadily during 2010. The grant support is sufficient for ongoing studies. The structure of the team is now optimized and the platforms in the lab are ready for more delicacy scientific works. Hopefully, we expect that more profound investigations could be done based on the ongoing KSHV and HBV studies, in order to find the potential antiviral strategies. We are confident that good work will be done in the next few years.

Presentations at national and international academic conferences

Deguang Liang

‘A KSHV encoded miRNA attenuates interferon signaling and contributes to viral latency maintenance by targeting IKKepsilon’

13th International Workshop on Kaposi’s Sarcoma Associated Herpesvirus (KSHV) and Related Agents, Los Angeles, August 29-September 1, 2010

Xianzhi Lin

'miR-K12-7-5p encoded by Kaposi's sarcoma-associated herpesvirus stabilizes the latent state by targeting viral ORF50/RTA'

13th International Workshop on Kaposi's Sarcoma Associated Herpesvirus (KSHV) and Related Agents, Los Angeles, August 29-September 1, 2010

Publications

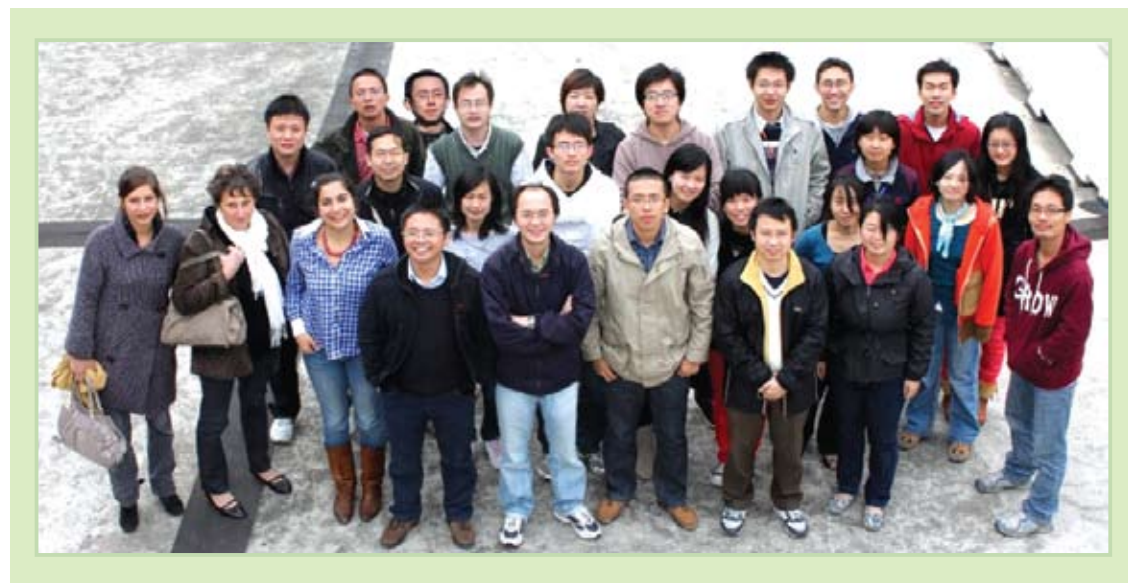
Lin X, Liang D, He Z, Deng Q, Robertson ES, Lan K*. miR-K12-7-5p encoded by Kaposi's sarcoma-associated herpesvirus stabilizes the latent state by targeting viral ORF50/RTA. PLoS One. 2011 Jan 20;6(1):e16224.

Liang D, Gao Y, Lin X, He Z, Zhao Q, Deng Q, Lan K*. A human herpesvirus miRNA attenuates interferon signaling and contributes to maintenance of viral latency by targeting IKK ϵ . Cell Res. 2011 May;21(5):793-806.

Zhu QY, Liu Q, Chen JX, Lan K, Ge BX. MicroRNA-101 targets MAPK phosphatase-1 to regulate the activation of MAPKs in macrophages. J Immunol. 2010 Dec 15;185(12):7435-42.

Wang X, Wang X, Liang D, Lan K, Guo W, Ren G. Classic Kaposi's sarcoma in Han Chinese and useful tools for differential diagnosis. Oral Oncol. 2010 Sep;46(9):654-6.

Wang X, He B, Zhang Z, Liu T, Wang H, Li X, Zhang Q, Lan K, Lu X, Wen H. Human herpesvirus-8 in northwestern China: epidemiology and characterization among blood donors. Virol J. 2010 Mar 17;7:62.



Unit of Viral Hepatitis (Established in 2007)

Principal Investigator

Jin Zhong

Ph.D.in Microbiology, University of Texas at Austin, USA (1997-2003)

Postdoctoral fellow at The Scripps Research Institute, USA (2003-2007)

Team members

Research Assistants: Yimin Tong, Na Wang, Yongfeng Gao, Qingchao Li

Unit Secretary: Cheng Cheng

Postdoctoral fellows: Yongfen Xu, Andy Tsun (in co-supervision with Unit of Molecular Immunology)

Ph.D students: Wanyin Tao, Rui Li, Ying He, Jie Lu, Qiang Ding, Yu Xiang, Xuezhi Cao

Co-supervised Ph.D student: Xiaogang Xiang (with Ruijin Hospital)

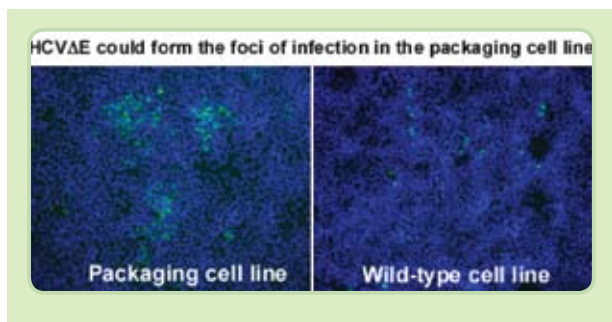
Research objectives

Hepatitis C virus (HCV) causes acute and chronic hepatitis, liver fibrosis and cirrhosis, and hepatocellular carcinoma. It currently infects more than 38 million people in China and 170 million people over the world. There is no vaccine to prevent HCV infection yet, and the current interferon-based treatment is partially effective and has strong side effects. The main interest of our laboratory is to study biology of HCV infection and virus-host interactions at the molecular and cellular levels. Our current efforts are being focused on the development of experimental model systems for HCV infection, identification of viral and host factors that determine the outcome of HCV infection in vitro and in vivo. We hope that our research will yield important findings that ultimately contribute to the prevention and treatment of HCV infection.

Highlights of Research Progress & Achievements

1. Develop a single-cycle infection cell culture model for Hepatitis C virus

A robust HCV cell culture infection system has been established using the JFH-1 isolate that allows the virus to efficiently propagate in tissue culture. The envelope glycoproteins are the major components of viral particles. Here we developed a trans-complementation system that allows for the production of infectious HCV (JFH-1) particles containing the envelope-encoding regions deleted genome. The cotransfection of the envelope gene-deleted JFH-1 RNA and a



plasmid that expresses JFH-1 E1 and E2 proteins in Huh7.5.1 cells resulted in the production of infectious HCV particles in the supernatant (denoted HCVΔE). The lack of envelope could also be complemented in packaging cells stably expressing the JFH-1 envelope proteins. HCVΔE production could be enhanced significantly by previously described adaptive muta-

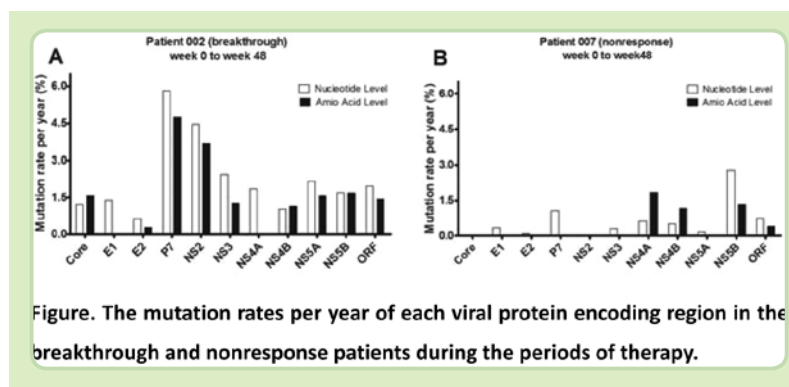
tions in NS3 and NS5A. Moreover, HCVΔE could be propagated and passaged in the packaging cells while only resulted in single-round infection in wild-type cells. Interestingly, we found that HCVΔE production could be efficiently rescued by vesicular stomatitis virus (VSV) glycoproteins, which no longer depended on host apolipoprotein E. The VSV glycoprotein-mediated viral entry could allow for the bypass of natural HCV entry process and the delivery of HCV replicon RNA into HCV receptor deficient cells.

This single-cycle HCV infection cell culture system should be an important addition to the current collection of cell culture models for studying the HCV life cycle and virus-host interactions. Because HCVΔE results in single-cycle infection and may likely induce both humoral and cellular immune responses after infection, our development provide a new potential way for the HCV vaccine development. This work has been published in *Journal of Virology*.

2. Study of viral sequence evolution in Chinese genotype 1b chronic hepatitis C patients experiencing unsuccessful interferon treatment

About 50% chronic HCV patients do not develop sustained virological response (SVR) to the standard interferon plus ribavirin treatment. Treatment failure occurs in the following forms: nonresponse (serum HCV RNA level declines less than 2 log between baseline and week 12, and remains above the detection limit throughout treatment), breakthrough (serum HCV RNA level drops below the detection limit, but rebounds while on therapy), and relapse (serum HCV RNA level drops below the detection limit, but rebounds after the therapy is discontinued). Unfortunately, molecular mechanisms underlying the failure of the interferon based therapy for chronic hepatitis C patients are still unclear. Both host and viral factors have been suggested to contribute to the failure of interferon based therapy. Breakthrough patients who initially respond to the interferon based therapy well and the HCV RNA level decreases to the undetectable level, but have reappearance of HCV RNA in serum during the therapy may represent a more unbiased subject to investigate whether the evolution of viral genomic sequences within a single patient play any roles in interferon resistance.

In collaboration with Professor Xie Qing at Ruijin Hospital of Shanghai, we have collected pre-, during- and post-treatment HCV-positive sera from patients who failed in the interferon plus ribavirin therapy. We have sequenced the entire



open reading frame of the HCV RNA genome that was isolated from a breakthrough patient whose serum HCV RNA levels decreased to below the detection limit but rebounded during the treatment and a nonresponse patient whose serum HCV RNA levels never decreased more than 2-log during the treatment. We found that the HCV genomic sequences in the breakthrough patient displayed significantly more mutations during the 1-year therapy than that in the nonresponse patient, with p7 and NS2 encoding regions having the highest mutation rate. The HCV sequence analysis of a 4-year post-therapy follow-up revealed that a vast majority of mutations selected during the therapy in the breakthrough patient were maintained while very few new mutations arose during the 4-year post-therapy span, suggesting the selection of the mutations during the therapy phase may be driven by the action of interferon and ribavirin and these adaptive mutations likely did not affect viral fitness.

Our study provided a new clue in understanding the interferon resistance during the therapy of chronic HCV patients. This work has been published in *Infection, Genetics and Evolution*.

3. Viral infection induces type III interferon production which can be blocked by hepatitis C virus NS3/4A protease.

Viral infection can trigger host innate immune response, and interferon production plays a critical role in the antiviral program and immune response regulation. IFN- λ , classified as type III interferon, could interfere with virus replication *in vitro* and *in vivo*. So far three IFN- λ s have been identified including IFN- λ 1, IFN- λ 2, IFN- λ 3 encoded by IL29, IL28A and IL28B, respectively. Three recent independent GWAS studies revealed a strong correlation of several single-nucleotide polymorphisms (SNPs) near and within the IL28B gene encoding IFN- λ 3 with sustained virological response of chronic HCV patients receiving the IFN- α and ribavirin combination therapy. One of these GWAS studies also identified a correlation between these SNPs and spontaneous clearance rate of acute HCV infection. However, the molecular mechanisms underlying these interesting genetic associations remain elusive.

Here we studied the biology of IL28 induction upon viral infection. We found that the IL28 ex-

pression rapidly increased after virus infection or double-stranded (dsRNA) RNA transfection in various types of cells, but not changed upon the type I and II interferon treatment, suggesting IL28 constitutes a primary and direct, rather than a secondary response to pathogens. Next, we demonstrated that virus infection or dsRNA transfection induced the IL-28 transcription via the NF- κ B and IRF3 mediated signaling pathways, requiring RIG-I, MDA5 and MAVS/IPS. Furthermore, the truncation analysis revealed a minimal IL-28 promoter region consisting of putative NF- κ B and IRF3 binding sites, which was sufficient for rendering the transcriptional regulation of IL-28 upon viral infection. Finally, we showed that HCV infection can inhibit polyI:C induced IL28 expression in Huh7 cells, and the NS3/4A protease activity was responsible for this inhibitory effect.

Our results presented important evidence for HCV suppressing host type III interferon response, and this finding should shed more light on the molecular mechanisms with which HCV thwarts host immune defense and establishes persistent infection. The manuscript on this work is currently under preparation.

Funding

- 11th Five-Year National Key Program on Infectious Diseases, MOH, China
- 973 program project, MOST, China
- NSFC General Project
- Chinese Academy of Sciences Key Projects
- TOTAL research grant on viral hepatitis
- Shanghai Pasteur Health Research Foundation
- Chinese Academy of Science 100-Talent Program
- French Pasteur ACIP Foundation

Collaboration

Intramural collaborators:

Bin Li, Bing Sun, Guangxun Meng, Paul Zhou, Yan Zhang, Tetsuya Toyoda, Zhong Huang, Rong Chen.

Extramural collaborators:

- Junqi Niu (No.1 Hospital, Jilin University)
- Qing Xie (Ruijin Hospital, Shanghai Jiaotong Univeristy)
- Yong-Jun Liu (MD Anderson Cancer Center, USA)
- Hongbing Shu (Wuhan University)
- Xiaolian Zhang (Medicine School, Wuhan University)

- Wei Yang (Institute of Pathogen Biology, CAMS)
- Yuanxu Zhang/Lai Ren (Kunming Institute of Zoology, CAS)
- Deyun Kong/Shuguang Wang (Shanghai Institute of Pharmaceutical Industry)

Publication

Li R, Qin Y, He Y, Tao W, Zhang N, Tsai C, Zhou P, **Zhong J.** (2011) Production of hepatitis C virus lacking the envelope-encoding genes for single-cycle infection by providing homologous envelope proteins or vesicular stomatitis virus glycoproteins in trans. *The Journal of Virology*, 85(5):2138-47. (Epub 2010 Dec 15)

Xiang X, Lu J, Dong Z, Zhou H, Tao W, Guo Q, Zhou X, Bao S, Xie Q, **Zhong J.** Viral Sequence Evolution in Chinese Genotype 1b Chronic Hepatitis C Patients Experiencing Unsuccessful Interferon Treatment. *Infection, Genetics, Evolution*, 2010,doi:10.1016/j.meegid.2010.11.011

Chen, F., Zhao, Y., Liu, M., Li, D., Wu, H., Chen, H., Zhu, Y., Luo, F., **Zhong, J.**, Zhou, Y., Qi, Z., and Zhang, X. (2010) Functional Selection of Hepatitis C Virus Envelope E2-Binding Peptide Ligands by Using Ribosome Display. *Antimicrob. Agents Chemother.*, 54, 3355–3364.

Weng, L., Hirata, Y., Arai, M., Kohara, M., Wakita, T., Watashi, K., Shimotohno, K., He, Y., **Zhong, J.** and Toyoda, T. (2010) Sphingomyelin Activates Hepatitis C Virus RNA Polymerase in a Genotype-specific Manner. *J. Virol.* 84, 11761-11770.

Perspectives

We will continue our research on HCV and focus on the following research direction:

- To study the functional roles of viral proteins in HCV life cycle particularly focusing on HCV proteins E1, E2 and NS3.
- To identify critical viral and host factors that determine the outcome of natural HCV infection and the outcome of interferon therapy of chronic HCV infection.
- To characterize the regulation of type III interferon induction upon viral infection.
- To develop experimental cell culture and animal models for HCV infection.

Presentations at invitation at national and international conferences

Jin Zhong

The 3rd China-Japan Science Forum, Wuhan, China, Mar.. 2010

“Hepatitis C Virus and Lipoproteins”

Jin Zhong

2010 Chinese Academy of Sciences Symposium on Infectious Diseases, Shanghai, China,
Nov.2010

“Transcomplementation of Hepatitis C Virus envelope glycoproteins”



Unit of Immunoregulation (Established on Dec.2007)

Principal Investigator

Qibin Leng

Ph.D in Hebrew University of Jerusalem Hadassah Medical School, Israel (2003)

Postdoctoral fellow at Immunology Department of Weizmann Institute of Science, Israel (Jan-Jul, 2003)

Postdoctoral fellow at Centre for Cancer Research of Massachusetts Institute of Technology, USA (2003-2007)

Team members

Laboratory secretary: Juexian Yi

Research assistant: Zhong Fang, Ruicheng Wei

Post-doctoral fellow: Mingfei Jin

M.S.-Ph.D students: Kai Zhu, Nining Guo, Na Zhang, Chunhui Yang, Juhao Yang, Jianrong Wang

Ph.D. student: Chunfu Yang

M.D candidate student: Bin Xu (Fudan University)

M.S. student: Qingsong Pan (Eastern China Normal University)

Intern: Nevine El Khatib

Research Interests

Although vaccines and drugs are important to ultimately prevent infectious diseases and cure infected patients respectively, frequently both of them are not immediately available when emerging viral infectious disease occurs. Thus, other interventional strategies are greatly appreciated to prevent disease spread and to mitigate disease severity of affected patients. We are studying the immunological factors that predispose the human individuals to be susceptible to emerging infectious diseases and affect disease severity. We hope to develop diagnostic tools for early detection of disease severity and new therapeutic and prophylactic approaches to cope with infectious diseases.

Research progress

1. Immune correlates of HFMD severity

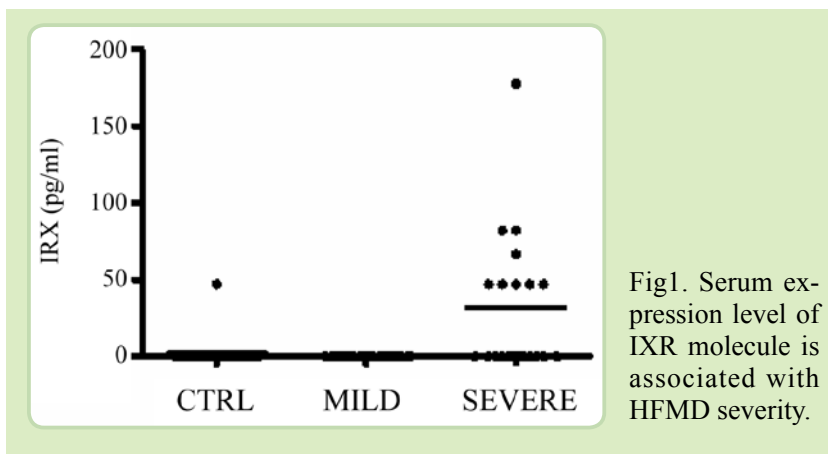
The hand, foot and mouth disease (HFMD) has emerged as a major infectious disease affecting children in China since March, 2008. Over 3.4 million cases have been reported according to the proceeding of the Ministry of Health of China so far. Majority of patients develop mild symptoms such as fever and blisters in the mouth and a skin rash, but some patients develop more severe neurologic symptoms and even die from pulmonary edema due to brainstem infection. Of note, severe cases and fatalities arise dramatically in 2010. However, what factors cause the current outbreaks and affect the clinical outcome of patients remains unclear. We carried out a retrospective case-control study on Fuyang HFMD outbreaks. We found that the proportions of children who had timely received poliovirus vaccine are 70.9%, 62.4% and 41.7% in health control, HFMD patients without pulmonary edema and HFMD patients with pulmonary edema, respectively [Table 1]. There was a significant difference in the proportion of children who had received the recommended OPV immunization between the group with edema and the controls. In addition, there was also a significant difference between the patients with edema and the one without edema, suggesting that the untimely poliovirus vaccination correlates to the HFMD severity, namely pulmonary edema.

Table 1. Regularly OPV immunization in HFMD patients with or without edema in comparison to control group during the 2008 HFMD outbreaks in Fuyang city

Groups	Regular Vacci. No. (ratio)	Odd Ratio (95% CI)	P value*
Controls (n=447)	317 (70.9%)		
All Cases (n=153)	88 (57.5%)	0.555 (0.380, 0.812)	0.0023
Without edema (n=117)	73 (62.4%)	0.680 (0.444, 1.042)	0.075
With edema (n=36)	15 (41.7%)	0.293 (0.146, 0.586)	<0.001
With vs without		0.431 (0.201, 0.921)	0.028

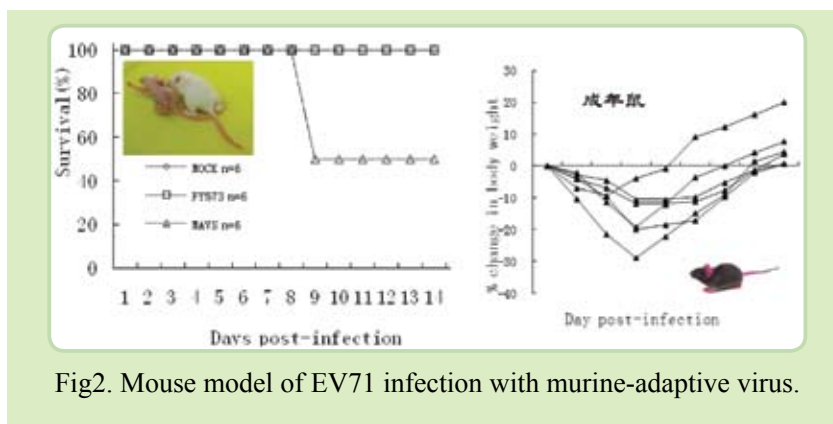
*Chi square (χ^2) test was used to calculate P value.

Currently, there is no vaccine or effective therapeutic against HFMD. Any diagnostic technology that can predict the HFMD severity will provide early and specific care to children susceptible to severe HFMD. We have performed protein array analysis of serum samples of HFMD patients and found that IRX was found only in the serum samples of severe HFMD patients but not in those of mild patients. In addition, the patients whose serum samples were positive to IRX had prolonged illness, fever and hospitalization. Thus most likely IRX can serve as a prognostic marker of HFMD severity.



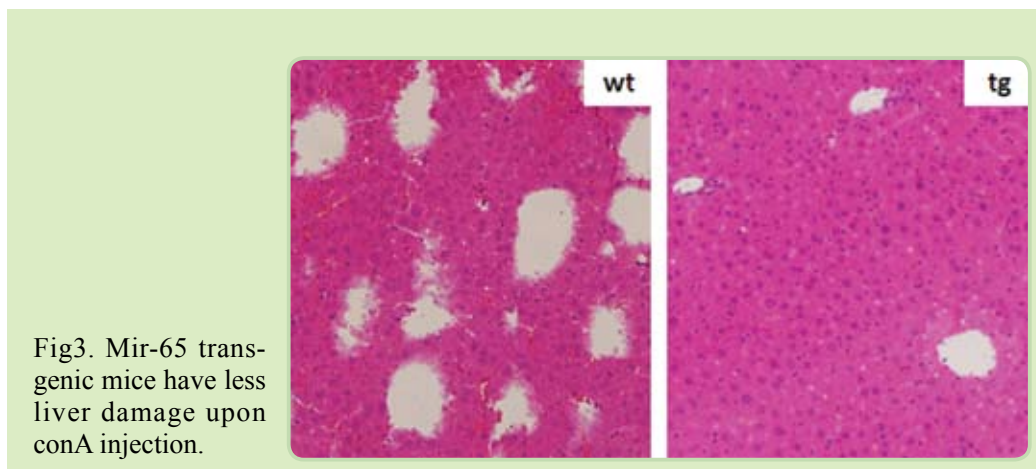
2. Adult mouse model of EV71 infection

The most feasible animal model for EV71 infection is neonatal or young mouse model. EV71 naturally does not infect mice, but it can infect less than two-week old mice after adaptation in the brain of neonatal mouse. We have succeeded in adapting a clinical EV71 virus isolate by repeatedly infecting neonatal mice and then culturing the virus in murine cells in vitro, and obtained a mouse-adaptive EV71 virus (MAV) that can be cultured and propagated in murine neuronal cells, suggesting the MAV has fully acquired ability to infect and replicate in murine neuronal cells. The MAV infects young ICR mice and causes the mice to develop neurological symptoms, such as weak limbs, paralysis, seizure and even death. Furthermore, when we infected X gene-knockout mice with the MAV, even 5 week-old mice were susceptible to the infection and developed symptoms, such as fluffy hair, weak limb and weight loss etc.. Since there is not a similar adult mouse model reported previously, the older mouse model of EV71 infections is of very important in studying immune responses and pathogenesis of EV71 infection and particularly evaluating the efficacy of EV71 vaccine.



3. Immune pathogenesis of fulminant hepatitis

Fulminant hepatitis usually results from a virus, toxin or immune mediated attack. Mortality without supportive management and/or liver transplantation is in excess of 70%. The processes leading to such profound hepatic damage remain not fully understood, but are multifactorial and depend on the age and susceptibility of the host and the extent of hepatic injury. We found that the mice transgenic for a newly identified MicroRNA, miR-65, had significantly reduced T-cell mediated hepatitis upon ConA injection (Figure 3). Similarly the mice deficient in IRX02, which is a known receptor expressing on leukocytes, also had dramatically reduced liver damage. In addition, it has been reported that the ligand of IRX02 was up-regulated by HCV infection. Thus, both Mir-65 and IRX02 are likely involved in T cell-mediated hepatitis caused by virus infection. While, interference of IRX02 signaling may be a promising drug target for treating fulminant hepatitis.



Funding

- SISEA grant for HFMD study.
- 973 National Key Project for Emerging Infectious Diseases.

Collaboration

- Drs Ralf Altmeyer and Zhong Huang, IPS.
- Dr Mei Zeng, Fudan University Children Hospital.
- Drs Junfeng Wan and Liyi Zhu, Fuyang CDC.
- Dr Chengfeng Qin, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology.

- Dr Dietmar Zehn, Swiss Vaccine Research Institute, Lausanne, Switzerland.
- Dr Honglin Xu, Beijing Institute of Serum and Biological products.
- Drs Hao Shen and Honglin Wang, Shanghai Jiaotong University Medical school.

Publication

Li Q, Ke F, Zhang W, Shen X, Xu Q, Wang H, Yu XZ, **Leng Q**, Wang H. Plasmin plays an essential role in amplification of psoriasiform skin inflammation in mice. **PLoS One**. 2011 Feb 2; 6(2):e16483.

Liu Q, Ku Z, Cai Y, Sun B, **Leng Q**, Huang Z. Detection, characterization and quantitation of Coxsackievirus A16 using polyclonal antibodies against recombinant capsid subunit proteins. **J Virol Method**, 2011 Feb 3. [Epub ahead of print]

Jiang T, Li X, Liu W, Yu M, Liu J, Yu X, Qin E, Cao W, **Leng Q**, Qin C. Serum Antibody Response to the Novel Influenza A (H1N1) Virus in the Elderly. **Clin Infect Dis**. 2010, 50:285-6.



Unit of Structural Virology (Established in 2007)

Principle Investigator

Rong Chen

Ph.D. from Baylor College of Medicine, USA (1997-2003);

Postdoctoral Associate, Baylor College of Medicine, USA (2003-2005);

Research Fellow in Microbiology and Molecular Genetics, Brigham and Women's Hospital, Harvard Medical School (2005-2006);

Research Fellow in Cell Biology, Harvard Medical School (2007).

Team members

Research Assistants: Yaling He, Jie Ding, Fuxiao Shen, Qian Wang

M.S-Ph.D Students: Ke Lv, XiaoXi Lin, ShuBing Tang

Ph.D Student: FuChun Yang

M.S Student: Haoyang Li

Research objectives

1. To elucidate the molecular and structural basis of EV71 pathogenesis

Enterovirus 71 or EV71, a member of the *Picornaviridae*, has caused significant morbidity and mortality in China and other Asia-pacific regions. As the major cause of severe hand-foot-and-mouth disease, EV71 were associated with a total of 353 deaths in 2009 and 890 deaths in 2010 in mainland China (China CDC). Currently, vaccines or drugs are still not available. We are interested to elucidate the molecular and structural basis of EV71 pathogenesis. Our first goal is to identify key host factors involved in EV71 pathogenesis through yeast two-hybrid experiments. We will continue by deciphering roles of these host factors involved in EV71 pathogenesis based on virology studies. In parallel we will carry out x-ray crystallography and cryoEM analysis to understand the structural details of various macromolecular complexes which are of importance to EV71 pathogenesis, such as virus with receptors or Fab of neutralization antibodies. We expect our studies will lead to a better understanding of the mechanism of EV71 pathogenesis, and provide clues for effective drug development.

2. To understand the structural basis of HCV cell entry

We are interested to elucidate the structural basis of HCV cell entry, through a hybrid approach including biochemistry and structural biology. HCV accomplishes its cell entry process via two viral glycoproteins forming as the E1E2 heterodimer, and at least four cellular receptors-CD81, claudin 1, SRBI and occludin. However, the detailed molecular events underlying HCV cell

penetration process is unclear. Two sets of experiments are carried out in parallel: 1) to produce large quantities of functional E1E2 heterodimer or functional VLPs with E1E2 incorporated; 2) to produce functional CD81, claudin 1, SRBI and occludin. We will then *in vitro* reconstitute various functional HCV cell entry complexes to elucidate the structural details, and to serve as model systems for HCV entry-inhibitor screening.

3. To produce 3D structural views of human paramyxoviruses

Both human respiratory syncytia virus and parainfluenza virus 3 are leading causes for respiratory tract illnesses. However, host factors important for their pathogenesis are barely known. We are in the process of identifying these host factors through yeast two-hybrid, GST-pull down and other related experiments. We will then carry out structural analysis of these identified host factors, either alone or in complex with viral components. Our goal is to produce structural views of various stages of viral pathogenesis with a hybrid approach of x-ray crystallography and cryoEM.

Highlights of achievements and progress

1. Identification of host factors involved in EV71-host interaction

Yeast two-hybrid approach has been taken to map the EV71 interactome. Various viral proteins encoded by the EV71 genome have been taken as prey to identify cellular proteins involved in EV71 pathogenesis. Particularly for VP1, a capsid protein, over 100 cellular proteins have been identified as interaction partners. For 2C, a non-structural protein involved in genome replication and one of the most conserved encoded by picornaviruses, at least 3 cellular proteins have been identified as interaction partners, among which one has already been reported by another group. We are currently carrying out siRNA experiments to understand the role of these host factors involved in EV71 pathogenesis.

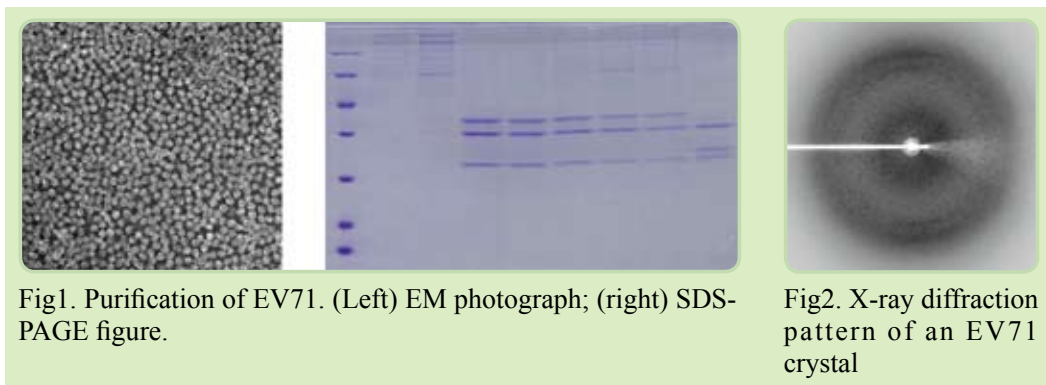
Further more, we are also interested to examine if these cellular proteins also interact with other picornaviruses, such as coxsackie virus A16 and poliovirus. Our preliminary results showed that about one third of interaction partners with EV71-VP1 also interact with poliovirus-VP1. Considering the fact that the capsid protein sequences are highly variable, our results suggest that there might exist host factors involved in broad-spectrum interaction with various viruses.

2. Structural characterization of EV71

We have produced VLPs in insect cells via the recombinant baculovirus system. In addition, we have purified large quantities of virions from either Vero or RD cells. Crystallization trays have been set up and we have obtained crystals from both VLPs and virions. Our recent trip to BL17U1, one of the beam lines at Shanghai Synchrotron Radiation Facility, has yielded a few sets of high-quality diffraction data. The resolution can get beyond 3.5 Å, which is sufficient for atomic-resolution structure determination.

In collaboration with the Academy of Military Medical Sciences, we have obtained neutralization antibodies against EV71. In addition, we are in the process of purifying EV71 receptors.

Future studies will be focused on structural characterization of EV71 or VLP in complex with neutralization antibody or identified cellular receptor.



Grants

- CAS 100 Talents Program, CAS (2010-2012)
- Frontier Research for Young Talents, SIBS-CAS (2008-2010)
- National Key Project on HCV, Ministry of Health (2008-2010)
- Shanghai PuJiang Career Development Award, Shanghai Municipality (2009-2011)
- 973 Project, MOST (2010-2014)

Collaboration (national and international)

- Dr. Chengfeng QIN, Academy of Military Medical Sciences
- Dr. Lingpeng CHENG, Institute of Biophysics, Chinese Academy of Sciences
- Dr. Jin ZHONG, Institut Pasteur of Shanghai, Chinese Academy of Sciences
- Dr. Jason JIANG, Cincinnati Children's Hospital, USA



Unit of Hematopoietic Stem Cell and Transgenic Animal Models (Established in 2009)

Principal Investigator

Yan Zhang

Ph.D. from Institute of Biochemistry and Cell biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (2001);

Postdoctoral Fellow at Department of Microbiology and Immunology, University of California, San Francisco(2002-2008)

Team Members

Unit Secretary: Xiaoli Yang

Research Assistant: Zhilong Wang, Xiujie Yuan, Xiaodan Ding

M.S. Students: Xiufeng Zhang(Shandong Normal University), Cuihong Zhou Peipei Zhou

Intern Students: Aiping Jiang (Huazhong Agricultural University), Feng Zhang(ZheJiang University), Xiaoyan Zheng (Zhengzhou University)

Transgenic Core Facility

Xiaogang ZHOU (Microinjection), Hui ZHANG (ES Cell Culture), Yiyi JIANG (Targeting Vectors Construction).

Research Objectives

- Investigating the functions of epigenetic regulators (Trithorax proteins, especially) in the development and maturation of the immune system.
- Generation of novel genetically engineered mouse models for virology, immunology and oncology studies.
- Establishment of Transgenic Core Facility in IPS.

Highlights of Research Progress & Achievements

1. Investigating the Roles of Trithorax Proteins in the Development of the Hematopoietic and Immune System

Polycomb group (PcG) and Trithorax group (TrxG) proteins are evolutionary conserved transcriptional repressors and activators that epigenetically modify chromatin, and play important roles in establishment and maintenance of different tissues in mammals. A number of gene-knockout mouse models have demonstrated the critical functions of PcG and TrxG genes in both mouse embryonic development and postnatal tissue homeostasis.

There have five TrxG members have been identified so far in mouse and human: *Mll1*, *Mll2*, *Mll3*, *Mll4* and *Mll5*. By generating gene-knockout mouse models, we and others have shown that *Mll* and *Mll5*, two *Trithorax* group (*Trx-G*) genes, play critical roles in regulating hematopoietic stem cell (HSC) self-renewal. Inactivation of these genes in mice lead to reduced cellularity of HSC compartment, which correlates with functional impairment of long-term repopulation potential. However, the molecular mechanism of *Mll1* and *Mll5* in regulating HSC self-renewal is uncertain. Also, it remains unclear whether other *Trx-G* group members, such as *Mll2*, *Mll3* and *Mll4*, have similar roles in HSC self-renewal regulation, and whether those *Trx-G* genes employ a common pathway to control HSC function.

Currently, no *Mll2* gene knockout mice has been reported; a *Mll3* partial deleted mutant (lack of C-terminal SET domain) mouse model has been shown to play vital role in adipogenesis; *Mll4* (old *Mll2*) conventional knock-out mice displayed embryonic lethality. No literatures so far described the roles of *Mll2*, *Mll3* and *Mll4* in the development of the hematopoietic and immune system. To address these questions, we sought to generate conventional and conditional gene-knockout mouse models of other *Trx-G* genes. We have generated *Mll2*, *Mll3* and *Mll4* gene-targeting vectors by using two-step recombineering. Both conventional and conditional gene-knockout mice can be generated by treating the targeted ES cells with *Cre* or *Flpe* expression plasmids. We have transfected *Mll2*, *Mll3* and *Mll4* gene-targeting vectors into mouse E14 ES cells. Three correctly targeted *Mll2* ES cell clones will be micro-injected into C57BL/6 blastocysts soon to generate germline-transmitted chimeras. The identification of correctly targeted ES cell clones for *Mll3* and *Mll4* are ongoing.

Despite the fact that *Mll5* plays critical roles in HSC self-renewal, the molecular mechanism is still unclear. To elucidate the molecular mechanism of *Mll5*, we begun to identify the proteins interacting with MLL5. Firstly, the 3×Flag-tagged human MLL5 protein were transiently overexpressed in HEK293 cells, then MLL5 complex were co-immunoprecipitated with anti-Flag antibody (M2) sepharose for further SDS-PAGE separation and Mass Spectrometry assay. A set of proteins were identified to be associated with MLL5, including proteins involved in transcription activation, DNA damage repair and epigenetic regulation etc. Right now, we are performing a series co-immunoprecipitation analysis to confirm the association. Meanwhile, we

performed yeast two-hybrid screening against human bone marrow and human fetal liver cDNA libraries for identifying MLL5 binding proteins, and a set of candidate MLL5 binding proteins were identified. We are now performing co-immunoprecipitation to confirm the binding of those candidate proteins with MLL5. Further experiments will be performed to dissect the biological significance of the interaction between MLL5 and those proteins.

Previous studies have shown that Hox genes are major downstream targets of PcG and TrxG genes. In our previous experiment, we found that inactivation of *Mll5* results in up-regulation of *Hoxb2* and *Hoxb5* genes in mouse HSC population. Since MLL5 protein may be part of chromatin remodeling complex and displayed H3K4 histone methyltransferase activity, we suspect that MLL5 protein could regulate downstream target genes transcription by binding to the regulatory region of the targets. To this end, we collaborate with Prof. Qianfei WANG in Beijing Institute of Genomics for genome-wide ChIP-Seq analysis. We sought to perform the ChIP-Seq assay with two strategies. First, 3×Flag-tagged human MLL5 protein were transiently overexpressed in HEK293 cells, then transfected cells were fixed by formaldehyde, then perform ChIP-Seq; Second, we sort out HSC population directly from wild-type C57BL/6 mice for fixation and precipitate with anti-MLL5 antibody, then perform ChIP-Seq analysis. We will use *Mll1* as control in parallel. We speculate that comparison of the genome-wide binding patterns between *Mll1* and *Mll5* will provide useful insight into the functions of TrxG group proteins.

2. Generation of Novel Transgenic and Knock-in Mouse Models for Virology, Immunology and Oncology Studies

(1) Generation of Human Receptors Transgenic and Knock-in Mouse Models for HCV and EV71 Infection Assays

Absence of ideal mouse models for Hepatitis C Virus (HCV) and Enterovirus 71 (EV-71) hampered the precise characterization of viral pathogenesis, vaccine development and antiviral drug discovery. Four membrane receptors: *CD81*, *Occludin*, *SR-BI*, and *Claudin1*, have been identified for efficient entry of HCV into human hepatocytes, and transfection of *CD81* and *Occludin* in mouse cells are sufficient for HCV entry. Similarly, two membrane proteins: *PSGL-1* and *SCARB2* have been identified as the receptors for the entry of EV71 into human cells.

Several previous studies have demonstrated that mice carrying human receptor genes provide a practical approach to generate mouse infectious models for human viruses. For example, mice carrying human *ACE2* receptor gene were susceptible to human SARS virus. Thus, we are trying to induce human *CD81*, *Occludin*, *PSGL-1* and *SCARB2* into mouse genome. We employ two strategies: first, we directly micro-inject human cDNA into mouse fertilized oocytes to generate transgenic mice carrying those four human cDNAs; second, we knock-in human cDNA into the mouse endogenous homolog loci by gene-targeting. Right now, we obtained five *PSGL-1* and *SCARB2* double transgenic founder mice, further viral challenge analysis will be performed by collaborating with Prof. Jin ZHONG, Qibin Leng and Zhong HUANG. We have

finish the construction of gene-targeting vectors for *CD81*, *Occludin*, *PSGL-1* and *SCARB2* genes, we performed ES cell transfection and positive ES clones for *CD81*, *Occludin*, *PSGL-1* and *SCARB2* genes have been obtained. We will microinject those ES clones into C57BL/6 blastocysts soon to generate germ-line transmitted chimeric mice. Then cross male chimeric mice with female C57BL/6 mice to generate human *CD81*, *Occludin*, *PSGL-1* and *SCARB2* knock-in mouse models for further HCV and EV71 viral challenge to evaluate the usefulness of those knock-in mouse models.

(2) Generation of Novel Tool Mouse Strains for Immunology Studies

Cre-Loxp based conditional or inducible gene knockout system are useful to determine the roles of specific gene in tissue- or cell type-specific manner. A number of *Cre* mouse strains have been generated to study the function of certain genes in the immune system in lineage-specific ways. For instance, *Lck-Cre*, *CD4-Cre* mice are widely used in deletion of genes in T cell lineage, *CD19-Cre* mouse is used in B cell lineage, *CD11c-Cre* mouse is used in dendritic cells, *CD11b-Cre* mouse is used in myeloid cells, *F4/80-Cre* mouse is used in mature macrophages, and so forth. However, there is no specific *Cre* mouse strain which could delete gene in NK or NK-T cells.

NK1.1 is a membrane surface marker specifically expressed on NK and NK-T cells, which frequently used for characterization of NK and NK-T cells by flow cytometry assay. We are then trying to generate a novel *NK1.1-Cre* knock-in mouse strain by inserting the *Cre* and *Cre-ER-T2* gene into the NK1.1 locus, by which the expression of Cre protein is under the control of mouse endogenous NK1.1(*Klrbl1c*) promoter. We have generated the *NK1.1-Cre* and *NK1.1-Cre-ER-T2* gene-targeting vectors and transfected into mouse ES cells. Several positive ES cell clone underwent homologous recombination at correct location have been identified. We microinjected those positive ES cells into C57BL/6 blastocysts and obtain more than five chimeric mice (>50% chimerism). Right now, we are crossing those male chimeric mice with female C57BL/6 mice to generate *NK1.1-Cre* and *NK1.1-Cre-ER-T2* mouse strains. After we obtain the *Cre* mice, we will cross to GFP reporter mice to evaluate the *NK1.1-Cre* and *NK1.1-Cre-ER-T2* mouse strains.

(3) Generation of Novel Chromosomal Engineered Mouse Model for Oncology Studies

Leukemia and lymphoma are malignant tumors in the hematopoietic and immune system. Chromosomal abnormalities including chromosomal deletion, inversion and translocation are frequently observed in leukemia and lymphoma patients. Mixed-lineage-leukemia caused by *Mll*-mediated chromosomal translocation is one kind of leukemia with poor clinical prognosis. More than sixty partner genes have been identified so far to be fused to *Mll*. Previous studies have shown that enforced expression of *Mll*-fusion proteins by using retroviral or lentiviral based vectors in mouse bone marrow cells resulted in rapid leukemogenesis in mice. In addition, transgenic or knock-in mice carrying *Mll*-fusion genes were reported to develop leukemia. Those mouse models provide invaluable tools to characterize the molecular mechanism of *Mll*-

induced leukemia. Nevertheless, the current mouse models could not faithfully mimic human disease progression.

To create new type of mouse models to mimic human leukemia patients, we are generating novel *Mll*-fusion translocated mouse models by using “**Chromosomal Engineering**” technology. Briefly, two loxp sites are inserted into specific loci into mouse genome by gene-targeting. With Cre recombinase, the combination between the two loxp sites will create a site-specific chromosomal translocation. We designed two half-GFP gene in these two gene-targeting vectors, and a functional GFP gene will form after the chromosomal translocation. Therefore, the cells underwent chromosomal translocation will become GFP positive, and could be detected and sorted by flow cytometry. This new leukemia mouse models will provide new insights into the mechanism of leukemogenesis and will provide a useful animal models for anti-cancer drug test.

3. Transgenic Core Facility in IPS

(1) Generation of gene-targeting vectors by using recombineering techniques

We have successfully established the recombineering system in the facility. Right now, we are able to make a gene-targeting (Knockout, Knock-in) constructs within one and half month.

(2) ES cell transfection and positive selection using long-PCR

We have successfully established the system for ES cell culture, ES cell transfection, ES cell positive clone identification by using long PCR assays. The Southern blotting system using non-radioactive methods is under the construction.

(3) Pro-nuclear DNA microinjection and Blastocyst ES cell microinjection

We have successfully established the pro-nuclear DNA microinjection and blastocyst ES cell microinjection in the facility. We have already obtain several transgenic founder mice and several chimeric mice with >50% chimerism.

(4) Mouse strains used in the facility

There are currently three mice strains used in the facility: C57BL/6J, DBA2, and ICR(CD-1), which are originally purchased from SLAC or B&K. We are use fertilized 0.5 dpc embryos from C57BL/6J/ DBA2/F1 mice for pro-nuclear DNA microinjection, 3.5 dpc embryos from C57BL/6J or C57BL/6J/ DBA2/F1 mice for blastocyst ES cell microinjection.

After breeding in our mouse room for one more generation, the quality of the mice is getting better. Nevertheless, we plan to directly induce all those mouse strains from Jax lab after we move into the new building and keep breeding those mice by ourselves.

Funding

- Knowledge Innovation Foundation (2009-2012)
- Shanghai Pasteur Health Research Foundation (2010-2012)
- SIBS Young Scientist Foundation (2009KIP210, 2009-2011)

- National Natural Science Foundation (30971672, 2011-2013)
- MOST 973 Project (2010CB945600, 2010-2014)
- “Stem Cell and Regenerative Medicine” Strategic Priority Research Program Project, CAS (XDA01010205, 2011-2020)

Collaboration

- Qianfei WANG, Ph.D. Beijing Institute of Genomics, Chinese Academy of Sciences.
- Tao CHENG, Ph.D. Institute of Hematology, Chinese Academy of Medical Sciences.
- Xuyu ZHOU, Ph.D. Institute of Microbiology, Chinese Academy of Sciences.
- Baidong HOU, Ph.D. Institute of Biophysics, Chinese Academy of Sciences.
- Nigel Killeen, Ph.D. Department of Microbiology and Immunology, University of California, San Francisco.
- Kevin Shannon, MD. Department of Pediatrics, University of California, San Francisco.

Publications

Zhang X, Zhou C, Gao H, Yuan J, **Zhang Y**. Transcriptional Regulation of Natural Killer and Natural Killer T Cell Development. *Chinese Bulletin of Life Sciences*. (2011) *In press*. (In Chinese).

Wong J, **Zhang Y**, Lieuw K, Tran M, Forgo E, Weinfurter K, Alzamora P, Kogan S, Akagi K, Wolff L, Le Beau M, Killeen N, Shannon KM. Use of Chromosome Engineering to Model a Segmental Deletion of Chromosome Band 7q22 Found in Myeloid Malignancies. *Blood*. (2010) 115(22):4524-32.



Unit of Viral Immunology (established in 2009)

Principal Investigator

Jianhua Wang

Ph.D in Virology from Kunming Institute of Zoology, CAS (2005)

Postdoctoral fellow at Medical College of Wisconsin, USA (2005-2009)

Team members

• Laboratory secretary: Haiyan Wang

• Co-PI: Haibo Wang, PhD

Postdoctoral fellow: Chanjuan Shen

Research assistant: Renrong Tian, Qianqian Guo, Jin-Feng Jiang

M.S.-Ph.D student: Yan Qin

M.S. student: Wan Liu, Yanhui Jia (in co-supervision), Rongrong Zhai (in co-supervision), Ji Zhang (in co-supervision)

Research Interests

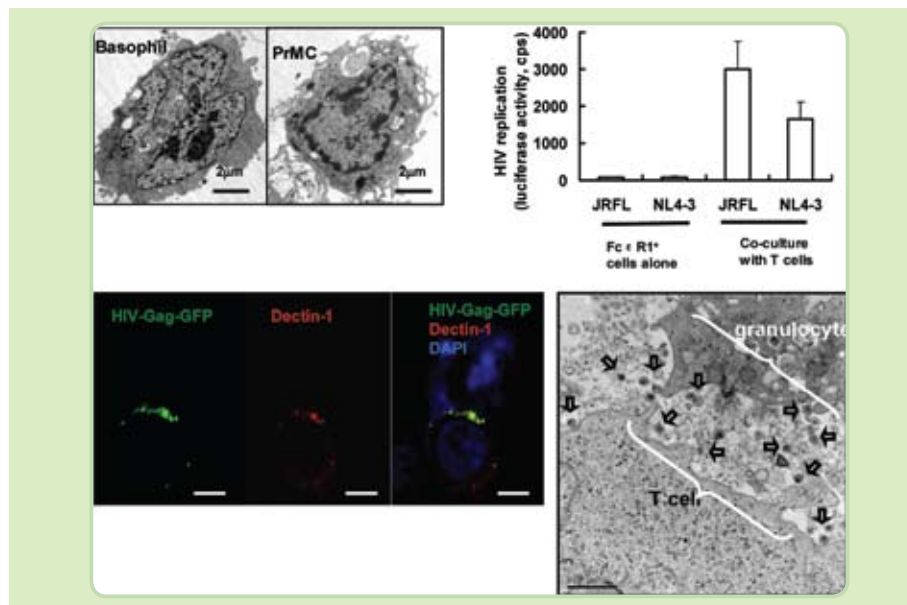
1. The interaction of HIV-1 with host cells and viral mucosal infection
2. The HIV-1 restriction in host cells
3. Co-infections and HIV-1 pathogenesis

Highlights of Achievements and Progress

1. Circulating FcεR1⁺ Granulocytes Mediate HIV-1 *trans* Infection of T cells

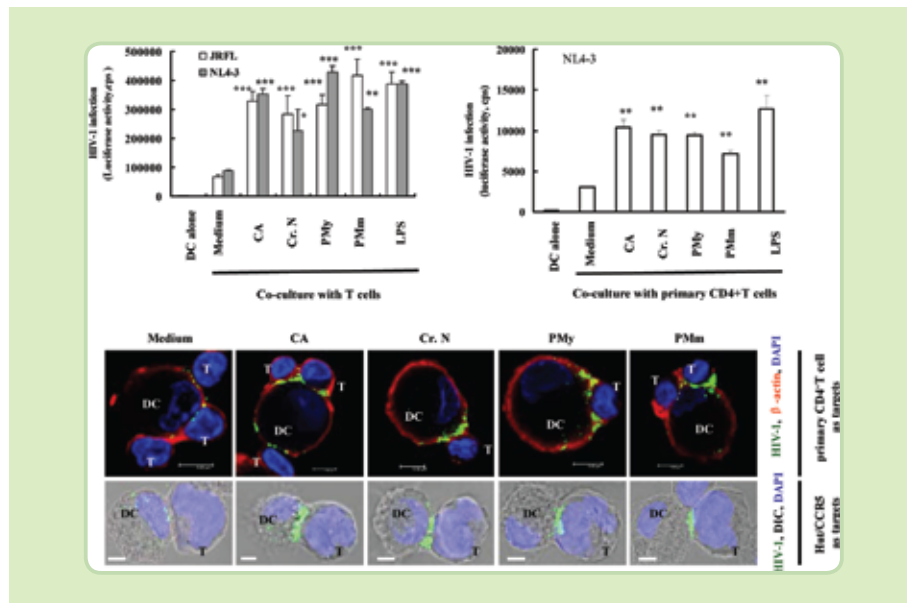
Human progenitor mast cells (prMCs) and basophiles are the specific cells in blood that expressing high-affinity receptors FcεRI. These granulocytes can be stimulated to degranulate for their best-known roles in the pathophysiology of allergic disorders. Accumulating reports support their essential contributions in host surveilling of invading pathogens such as parasites and bacteria, while the involvement of prMCs and basophiles in viral infections is enigmatic and less studied. Several literatures have mentioned the susceptibility of prMCs to HIV-1 infection due to surface expression of viral receptors. But the prMCs become resistant to new infections after migration to tissue compartments and maturation. Human tissue mast cells are an inducible reservoir of persistent HIV infection. However, the role of mast cells in HIV infection is still obscure. Here we show for the first time that the circulating prMCs and basophiles can

capture HIV-1 and efficiently transmit the virus to encountered CD4⁺T cell, facilitating viral dissemination. Dectin-1, a surface expressed C-type lectin, mediates HIV capture and transfer. Prior blocking via anti-Dectin-1 monoclonal antibodies or specific siRNA interfering of surface expression can dramatically impair HIV capture and the following viral transmission. Cell-cell direct contact between these FcεRI⁺ granulocytes and CD4⁺T target cells to form the virological synapses is required for viral efficient transfer. Our results figure out the novel potential role of circulating FcεRI⁺ prMC and basophiles in HIV dissemination, facilitating the understanding of the complicated HIV primary infection events, and probably provide additional clue against viral mucosal infection.



2. The modulation of miRNA on HIV-1 infection in monocytes

Monocytes are the precursor cells of a variety of immune cells such as dendritic cells and macrophages and play important role in HIV-1 infection and pathogenesis. Monocytes show restriction for HIV-1 replication and the constraint will be lost when differentiate into dendritic cells or macrophages. Varied mechanisms have been described, the lacking of the HDFs (HIV-1 dependent factors) was one of the reason. By analyzing the data from mRNA array in monocytes compared with DCs, we identified the candidate gene of pur-alpha, an HDF, and the differently expressed miRNA targeted pur-alpha was also focused on. By analysis of the modulation of miRNA on HIV-1 infection in monocytes, we hope to facilitate the understanding of HIV-1-host cells and try to find the new clue for antiviral strategies.



Grants

- ‘100 Talent Program’, Chinese Academy of Sciences
- Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-YW-R210, KSCX2-EW-Q-2)
- Knowledge Innovation Program of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences
- Shanghai Natural Science Foundation
- Shanghai Rising-Star Program (A type)
- Open Research Fund Program of the State Key Laboratory of Virology of China
- Sanofi-Aventis-SIBS Scholarship Program.

Collaboration

Extramural collaborators:

- Chiyu Zhang (Jiangsu University);
- Jifu Wei (The people’s hospital of Jiangsu province)
- Hongxiong Guo (Jiangsu province CDC)
- Yuye Li (Medical College of Kunming, Yunnan)
- Baochi Liu (Fudan University)
- Yongtang Zheng (Kunming Institute of Zoology, CAS)

- Rongge Yang (Wuhan Institute of Virology, CAS)
- Stephen Tsui (Chinese University of HongKong)
- Li Wu (the Ohio State University, USA)

Intramural collaborators:

- Lan Ke, Zhong Huang, Hui Xiao

Perspectives

The research aim of this unit will continue to focus on the interaction of HIV-1 and host cells, and try to better understand: 1) the mechanisms underlie viral mucosal infection, via the ex vitro and in vivo models. 2) the host factors that restrict the HIV-1 replication in monocytes, and the relative regulation of HIV-1 dependent factors by host microRNA; 3) The activation of dendritic cells by co-infected pathogens, and the following enhanced viral replication and viral dissemination.



Unit of Molecular Immunology (Established in 2009)

Principal Investigator

Bin Li

Ph.D. in Botany from Peking University (2001),

Postdoctoral Fellow (2001-2006) and Research Associate (2006-2009) in Immunology at the University of Pennsylvania School of Medicine,

Research Visiting Scholar in Immunology in Sir William Dunn School of Pathology at the University of Oxford (2002-2003)

Team members

Research assistant: Fang Lin, Zhiyuan Li, Jing Zhang, Kongchen Li

Post-doctoral fellow: Yongfeng Xu, Andy Tsun

Ph.D. students: Yayi Gao, Chen Chen

M.S-Ph.D. students: Zuojia Chen, Zhao Shan, Jia Nie, Yangyang Li, Jing Yang

M.S. students (Co-supervising): Zhimei Gao

Ph.D. students (Co-supervising): Augusta Nsonwu-Anyanwu (supported by TWOWS Fellowship), Jinsong Su

Trainee: Xiaohua Zhou, Evelyn Ly, Yue Xing, Zihou Deng, Ke Liu, Mengran Qian, Marco Pimentel, Sheng Li, Minjun Ni, Shuying Yin, Fujia Yao

Research objectives

Our research goal is to elucidate the cellular and molecular mechanisms regulating immune responses in health and disease including infectious diseases, autoimmune diseases and cancer. Currently, we are particularly interested in understanding the dynamic role of a subpopulation of T cells, namely CD4+CD25+FOXP3+ regulatory T cells (FOXP3+Tregs) in major human infectious diseases.

Activity of the forkhead family transcription factor Forkhead Box P3 (FOXP3) determines the immune function of FOXP3+Tregs. Upon infection, FOXP3+Tregs may suppress effector immune cell responses leading to the failure of clearing infection. FOXP3+Tregs may also help to limit collateral tissue damage during heightened inflammation. Furthering our understanding the regulation of FOXP3 and the dynamic ensemble of enzymatic cofactors in Tregs can thus pro-

vide therapeutic clues on how to control major diseases such as HIV, hepatitis B and C viruses.

Highlights of achievements and progress

Our group currently runs five main projects, two of which have made significant progress and are at a near publication stage.

(1) The underlying molecular mechanism of E3 ubiquitin ligase STUB1, a negative modulator of FOXP3 function: This project explores how numerous environmental “danger signals” generated during inflammation can modulate FOXP3+Treg cell function. Ubiquitination is a crucial pathway of cell-mediated protein degradation. Zuojia Chen has discovered that a stress-signal activated E3 ubiquitin ligase STIP1 homology and U-box containing protein 1 (STUB1) can interact with FOXP3, promoting its polyubiquitination and degradation *in vitro* and *in vivo*. We have already presented these data during two international conferences and are at the submission stage for this project.

(2) DBC1 is a Novel Binding Partner of FOXP3: Yayi Gao has generated a FOXP3-expressing stable cell line used for the purification of the FOXP3 transcriptional complex in T cells. Our Mass Spectrometry sequence results reveal a previously unidentified interaction partner of FOXP3 named Deleted in Breast Cancer 1 (DBC1), as a subunit of the FOXP3 complex. It seems that DBC1 does not dramatically affect the stability of FOXP3, but competes with the lysine deacetylase SIRT1 in binding to FOXP3. Our findings suggest that DBC1 could be involved in maintaining the acetylated form of FOXP3, which is crucial for the physiological function of FOXP3 in Treg cells. We propose that DBC1 acts as a positive regulator of FOXP3 by inhibiting SIRT1 mediated deacetylation of FOXP3, which could represent a new molecular pathway in modulating FOXP3+Treg function in health and disease.

(3) USP21 is Novel Binding Partner of GATA3 and Positively Regulates the Stability of GATA3: Regulatory T cells (Tregs) were originally discovered as immune suppressors critical for self-tolerance and immune homeostasis. Tregs were recently shown to possess plasticity to convert into effector T cells under certain stimuli. GATA3 expression in Tregs can lead to their conversion into T-helper 2 cells (Th2) *in vivo*, unlike the differentiation from naïve CD4 T cells where GATA3 expression is dependent on STAT6. However, the underlying mechanisms remain undefined. In our studies we found that after the stimulation via the T cell receptor, USP21 expression is up-regulated by FOXP3. Furthermore, USP21 can interact and stabilize GATA3 by acting as a GATA3-targeted deubiquitinase. The USP21 C221A mutant, however, loses the ability to stabilize GATA3. Knockdown of USP21 in FOXP3-expressing T cell stable cell lines leads to the downregulation of GATA3 expression in these cells. Thus, we have identified a FOXP3-dependent USP21-mediated mechanism that favors Treg-Th2 conversion.

(4) The Underlying Molecular Mechanism of IFITM3 as an Antiviral Restriction Factor:

In order to investigate the anti-viral properties of interferon-induced transmembrane protein 3 (IFITM3) as a host restriction factor, we purified the IFITM3 complex using the Tandem Affinity Purification (TAP) system in TAP-IFITM3 U2OS stable cell lines. After SDS-PAGE and silver staining, specific bands were sent for Mass Spectrometric (MS) analysis. These MS readings showed that IFITM3 can interact with CCT members (chaperonin containing T-complex polypeptide (TCP1), also called TRiC (TCP1 ring complex)). We then confirmed their interaction via co-immunoprecipitation. Moreover, these MS results reveal that K88 of IFITM3 is monomethylated. Through sequence alignment, we have predicted several potential methyltransferases of IFITM3. Our preliminary results suggest that IFITM3 may limited viral infection in a K88-monomethylation dependent manner..

(5) Mechanisms Underlying Post-translational Modification of MUM1/IRF4 and Mediated Transcriptional Regulation in the Immune System:

We have successfully generated the Tandem Affinity Purification-interferon regulatory factor 4 (TAP-IRF4) Jurkat cell line and purified the complex via the TAP two-step pull-down method. We have identified several monomethylation sites on IRF4 protein by Mass Spectrometry and verified the interaction between IRF4 and human FOXP3. A point mutation on the monomethylation site (K123) prevents IRF4 from localizing to the cytoplasm, but has no effect on its interaction with FOXP3. Finally, our recent data have identified one lysine methyltransferase specifically interacts with IRF4.

Current Funding

Bin Li (PI)

- CAS “100-talent program” (2009.1-2012.1)
“The role of FOXP3+ regulatory T cells in human major infectious diseases”
- NSFC General Project (Grant No. 30972702) (2009.1-2012.1)
“Mechanism studies of FOXP3 posttranslational modification and complex ensemble in regulatory T cells”
- The Science and Technology Commission of Shanghai Municipality Grant (Grant No. 09JC1416100) (2009.9-2012.8).
“Mechanism studies of TLR signal pathway mediated regulation of FOXP3 activity”
- The Shanghai Science and Technology Rising-Star Program 04/01/2010 – 3/31/2012 (Grant No. 10QA1407900)
“Mechanistic studies of IL-6 signal pathway mediated regulation of FOXP3 activity”
- CAS-Novo Nodisk Foundation (Grant No. NNCAS-2009-5 (SIBS)) (2010.1-2012.1)
“To evaluation of GITR-GITR-L interaction in human immune system as a therapeutic target for autoimmune diseases”.

- Shanghai Pasteur Health Research Foundation (Grant No. 09JC1416100) (2009.10-2010.9)
“Mechanism studies of FOXP3+ Treg cells in chronic HCV infection”
- The CAS network lab on Treg and novel vaccine development (Grant No. KSCX2-YW-R-206) (2010.1-2011.12)
“Development of FOXP3 inhibitors as potential vaccine adjuvants”

Bin Li as co-PI:

Zheng SG (Co-PI Bin Li)

- Tina C Foundation/Lupus Research Award, Arthritis Foundation (2010.7-2012.6)
“The ability of TGF- β to induce Foxp3+ regulatory T cells in lupus cells”.

Other lab members as PI:

Zhengju Yao

Frontier Research for Young Talents, SIBS-CAS (2010.8.2012.7)

The molecular mechanisms of post-translation modification and dynamic ensemble of transcription complex of ThPOK

Andy Tsun

CAS Fellowship for Young Foreign scientist and Funding supported by NSFC.(2010.7-2011.7)
“The regulation of Foxp3+ regulatory T-cells (Foxp3+ Treg) in chronic Hepatitis C Virus (HCV) infection in humans”

Collaboration (national and international)

Collaboration within IPS:

Jing Zhong, Bing Sun, Yan Zhang, Ke Lan, Jianhua Wang, Ralf Altmeyer

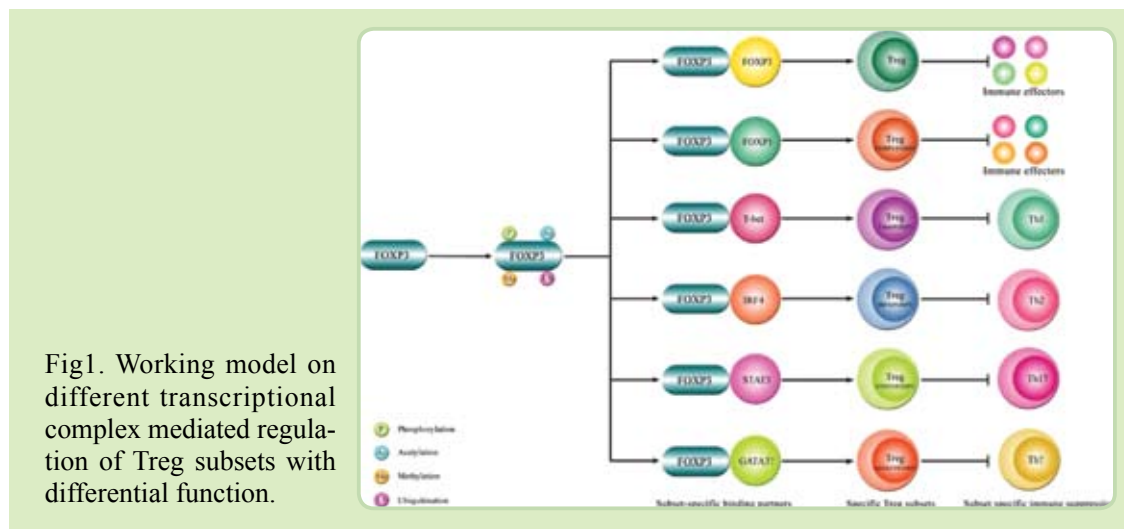
Collaboration Outside IPS:

- Karin Loser (The university of Munester, Germany)
- Fan Pan(John Hopkins University, USA)
- Mark Greene (University of Pennsylvania, USA)
- Song-Guo Zheng (University of South California, USA)
- Baochi Liu (The Shanghai Public Health Clinical Center)
- Guochao Shi (Ruijing Hospital)

Perspective

Recent findings suggested that key transcription factors involved in Th cell differentiation, such as T-bet, IRF4, GATA3, ROR γ t and STAT3, are also expressed in FOXP3+Treg cells, which could determine the specificities of differential subsets of FOXP3+Treg mediated im-

immune regulation. We intend to comprehensively identify the differential subunits of the transcriptional complex in different FOXP3+ Treg subsets, and reveal their functional relevance to FOXP3+Treg cell mediated immune regulation in health and disease (Figure 1).



Presentations at invitation at national and international conferences

Jing Zhang

Mechanism underlying Deubiquitinase USP21 mediated positively regulation of GATA3
7th Congress of the Chinese Society for Immunology, Beijing, October 25, 2010

Zuojia Chen

The 2nd International Conference on Regulatory T Cells and Th17 Cells and Clinical Application in Human Diseases. Shanghai, July 18, 2010

“Identification of STUB1 as an ubiquitin E3 ligase of FOXP3” (Oral presentation)

Bin Li

The Novo Nordisk Beijing Scientific Forum, Beijing, Nov.3, 2010

“Mechanism underlying FOXP3+ Treg cells mediated immunosuppression”

Bin Li

The 2nd International Conference on Regulatory T Cells and Th17 Cells and Clinical Application in Human Diseases. Shanghai, July 18, 2010

“Structural insights into the regulatory function of discrete acetylated residues of the FOXP3 coiled coil subdomain in oligomerization-dependent immune modulation”

Bin Li

The 3rd HKU-Pasteur Immunology Course, Institut Pasteur of Hong Kong, Nov.10. 2010
“Treg cells”

Publications

Chen ZJ*, Barbi J*, Yang HY, Li ZY, Gao YY, Dang EV, Jinasena D, Zheng Y, Fu J, Lin F, Chen C, Zhang J, Yu N, Li X, Shan Z, Nie J, Gao Z, Li YY, Yao ZJ, Tsun A, Luo WB, Semenza GL, Zheng SG, Loser K, Greene MI, Drew M., DM, Pan F** and **Li B**** . STUB1 negatively modulates regulatory T cell suppressive activity by promoting FOXP3 degradation. **In submission.** (* co-first authors; ** co-corresponding authors)

Chen ZJ, Lin F, Gao YY, Li ZY, Xing Y, Deng ZH, Yao ZJ, Tsun A and **Li B** * (2010) FOXP3 and ROR γ t: Transcriptional regulation of Treg and Th17. *International Immunopharmacology*. 2010 Nov 13. [Epub ahead of print] PMID: 21081189

Xiao Y, **Li B**, Zhou Z, Hancock WW, Zhang H, Greene MI (2010) Histone acetyltransferase mediated regulation of FOXP3 acetylation and Treg function. *Current Opinion In Immunology*. 2010 Sep 23. [Epub ahead of print] PMID: 20869864

Chen ZJ, Li ZY, Gao YY, **Li B** (2010) FOXP3+ regulatory T cells. *Chinese Bulletin of Life Sciences* Vol. 22, No.6, page 515-528



Unit of Vaccinology & Antiviral Strategies

(Established in 2009)

Principal Investigator

Zhong Huang

Ph.D. from China Agricultural University (1997)

Postdoctoral Fellow at Boyce Thompson Institute at Cornell University, USA (1998-2002)

Faculty Research Associate at Arizona State University, USA (2002-2005)

Assistant Research Professor at Center for Infectious Diseases and Vaccinology, Arizona State University, USA(2005-2008)

Team Members

Research assistants: Bo Wang, Xulin Huang, Jinping Shi

Lab Secretary: Jiejun Wen

Ph.D. student: Qingwei Liu, Yicun Cai

M.S. students: Xiaohua Ye, Fei Liu, Yanfang Feng, Ting Wang, Dapeng Li, Zhiqiang Ku

Objectives

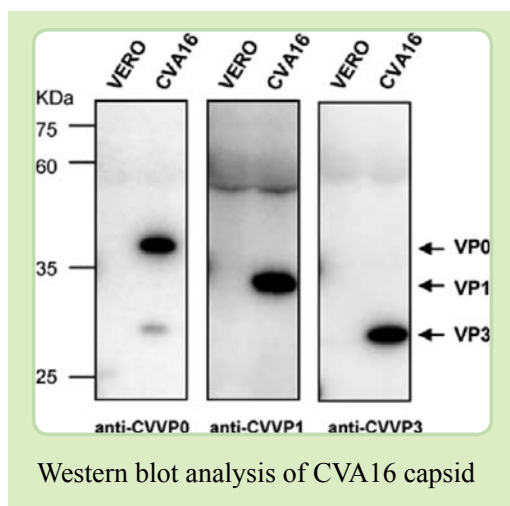
The main interest of our laboratory is to develop novel vaccines and immunization strategies for important viral diseases.

Research Progress

Hand, foot, and mouth disease (HFMD) is a common illness in children. However, no specific vaccine or antiviral drug is yet available for HFMD. Coxsackievirus A16 (CVA16) and enterovirus 71 (EV71) are two major etiological agents of HFMD. Currently, our research focuses on developing safe and effective vaccines against both EV71 and CVA16.

1. Generation of protein standards and polyclonal antibodies for detection, characterization and quantification of CVA16 and EV71

We have studied the prokaryotic expression of capsid subunit proteins and the generation of corresponding polyclonal antibodies of CVA16 and EV71. We showed that these recombinant



proteins and antibodies can be utilized to detect, characterize and quantify CVA16 and EV71, hence providing reagents and methods for qualitative and quantitative determination of the virus during the development of diagnostics and vaccines.

2. Development of a virus-like particle vaccine against CVA16

Virus-like particles (VLPs), which are formed by self-assembly of recombinant viral structural proteins from many viruses, are structurally and antigenically indistinguishable from the parental infectious virus, but lack viral nucleic acid and

are therefore absolutely noninfectious. VLPs have emerged as a safe and effective strategy for development of vaccine against viruses. We reported for the first time the generation of recombinant CVA16 VLPs and their ability to induce a high-titer neutralizing antibody response in mice. CVA16 VLPs were produced in insect cell by a recombinant baculovirus co-expressing P1 and 3CD. Biochemical and electron microscopy analyses revealed that the VLPs consisted of VP1, VP3, VP0, and to a lesser extent, VP2 capsid subunit proteins, and were present in a ~25nm spherical particle form. Immunization with CVA16 VLPs induced capsid subunit protein-specific antibody responses in mice. More importantly, VLP-immunized mouse sera efficiently neutralized live CVA16 virus with high titers. Collectively, our results indicated that CVA16 VLPs have strong protective potential against CVA16 and should be included in formulating a safe and effective bivalent HFMD vaccine against both EV71 and CVA16.

3. Towards a universal EV71 vaccine

EV71 can be divided into 11 subtypes which have been undergoing rapid evolutionary changes. Therefore, a successful EV71 vaccine must provide protection against a broad spectrum of EV71 strains/subtypes. SP55 and SP70, two conserved neutralizing epitopes within VP1 protein of EV71, have been identified. However, as small peptides, they are poorly immunogenic. We hypothesized that the immunogenicity of SP55/SP70 could be greatly enhanced by high-density presentation on VLPs. We designed and expressed hepatitis B core antigen (HBcAg) fusions with SP55/SP70 in *E.coli*. The resultant fusion proteins assembled into chimeric VLPs with SP55/SP70 displayed on the surface. Immunization with the chimeric VLPs induced SP55/SP70-specific antibody responses in mice. More importantly, the antisera efficiently neutralized a panel of EV71 strains isolated at different locations and years. Our data suggest that chimeric VLPs presenting SP55/SP70 are excellent candidates for a universal EV71 vaccine.

Collaborators

- Paul Zhou, Jianhua Wang, Bing Sun, Qibin Leng, Yan Zhang, Rong Chen at IPS-CAS
- Wei Liu (Guangxi CDC, China)
- Zhiguo Su (Institute of Processing Engineering, CAS)
- Wenqi An, Xiaojun Lin (HUALAN BIO)

Funding

- National 11-5 Key Program Grants on infectious diseases
- CAS “100 talents” Program
- 2010 SA-SIBS scholarship

Patent Applications

- “A universal vaccine against enterovirus 71”, application # 201010548081.0
- “A recombinant coxsackievirus A16 like particle vaccine”, application # 201010553419.1

Publications (*corresponding author)

Feng Y, Liu Q, Ku Z, Wen J, Shan H and Huang Z* (2011) Expression of VP0 protein of enterovirus 71 in E.coli and generation of corresponding polyclonal antibodies. *Chinese Journal of Cellular & Molecular Immunology* (accepted)

Liu Q, Ku Z, Cai Y, Sun B, Leng Q and Huang Z*. (2010) Detection, characterization and quantification of coxsackievirus A16 using polyclonal antibodies against recombinant capsid subunit proteins. *Journal of Virological Methods* (in press)

Huang Z, Phoolcharoen W, Lai H, Piensook K, Cardineau G, Zeitlin L, Whaley KJ, Arntzen CJ, Mason HS and Chen Q. (2010) High-level rapid production of full-size monoclonal antibodies in plants by a single-vector DNA replicon system. *Biotechnology & Bioengineering*. 106(1):9-17.



Immune Signaling and Regulation (Established in 2010)

Principal Investigator

Hui Xiao

Ph.D. in Molecular Genetics from Shanghai Institute of Plant Physiology (1994-1997)

Postdoctoral fellow, Dept. of Medicine, Case Western Reserve Univ., U.S.A. (1998-2002)

Team members

Research assistants: Aiping Zang, Huanjing Shi, Qi Liu

Research secretary: Jing Mu

Students: Zihou Deng, Yuan Gao, Shixin Ma

Research Objective

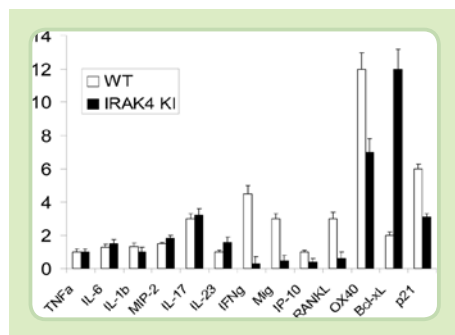
Toll-like receptors (TLRs) superfamily comprises a large group of receptors, which have evolved to recognize a broad range of molecular signatures associated with microbes. By sensing the presence of invading pathogen or danger signals released by damaged tissue, TLRs activate highly conserved signaling network, whereby lead to the induction of a plethora of genes involved in inflammation, immune regulation or tissue remodeling. Despite TLRs play a pivotal role in host defense, inappropriate activation of TLR signaling has been associated with a variety of human diseases, including chronic inflammation and cancer. Nevertheless, the precise mechanisms underlying the role of TLRs in host defense and inflammation-associated diseases are largely unclear. The objectives of our research are aimed at uncovering the molecular mechanisms by which TLRs recognize pathogen, initiate inflammation and tailor adaptive immune response, thereby providing novel insights into development of new vaccines and treatment of virus-induced chronic inflammation and cancer. We will be primarily focusing on three lines of studies: 1) Deciphering the signaling pathways by which TLRs regulate the expression of proinflammatory genes from transcription level to post-transcriptional level, providing new insights into constrain of inflammatory conditions. We will particularly research on the kinases which regulate the transcription, stability and translation of mRNA. 2) Identifying the mechanisms by which TLRs cross-talk with NLRs and RLRs in pathogen recognition as well as immune evasion mechanisms exerted by viruses, exploring novel vaccination strategies. 3) Using gene modified

mice model to deeply understand the roles of TLRs in anti-virus infection and tumorigenesis; then, validating animal models for screening and testing anti-cancer drugs.

Highlights of achievements and progress

1. Determine the mechanisms by which TLR regulates colitis and cancer

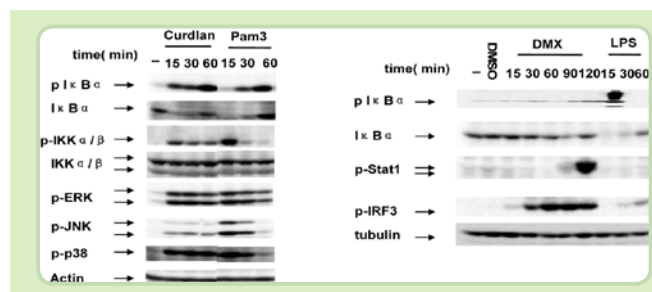
We have previously reported that TLRs/IL-1R signaling plays a critical role in maintaining gut homeostasis, controlling inflammation and suppressing tumorigenesis. To further determine the mechanisms by which TLRs and IL-1R regulate inflammation and tumorigenesis, we applied DSS-induced colitis model to B6 mice untreated or treated by IRAK4 kinase inhibitor. Compared to the untreated mice, IRAK4 kinase inhibited mice exhibited extensive tissue damage and much severer colitis, largely attributing to their incapability of repairing damaged epithelium. Furthermore, AOM+DSS administration induced more colon tumors in mice treated by



IRAK4 inhibitor, most of which had highly malignant tumors invading into the submucosa. Real-time PCR uncovered that decreased IFN γ and IP-10 expression might result in compromised anti-tumor immune response, contributing to increased tumorigenesis in IRAK kinase inhibited mice. As IRAK4 is an upstream kinase leading to the activation of mTOR, blockade of mTOR by rapamycin is being tested for its role in colitis-associated cancers.

2. The synergistic effect of TLR and non-TLR ligands in immune regulation

To test the synergistic effect of TLRs and non-TLRs in priming adequate immune responses, we studied the signaling mechanisms of TLR2 and dectin-1. Despite IRAK4 and IRAK1/2 are essential components for TLR2 signaling, they are not required for dectin-1 ligand curdlan-induced activation of NF κ B and MAPKs IRF3. By real-time PCR, we found that TLR2 ligation induced robust IL-12 expression, contributing to the polarization of Th1 response. In contrast, dectin-1 engagement led to IL-23 and IL-10 expression, but very little IL-12 production, resulting Th17 and Th2 response. By co-stimulating macrophages with Pam3 and Curdlan, higher production of IL-12, IL-23 and IL-17 were induced, supporting an anti-viral Th1/Th17 response. As proinflammatory cytokine TNF α was not further induced, these data implicates that curdlan/Pam3 could be used as adjuvants for vaccination. Further studies are under way to assess the combined



formulation of these non-TLR/TLR synergism in EV71 vaccine.

Grants

- NSFC General Project, (.2011.1 to 2013.12)
“The research on mechanisms of mTOR to regulate cancers which related to inflammation response in TLR and IL-1R signaling”

Collaboration

Internal

Paul Zhou, Jianhua Wang, Yan Zhang, Zhong Huang.

External

- Yiming Shao (CDC)
- Xiaoxia Li, Bin Zhang (Cleveland Clinic Foundation, USA)
- Aimin Jiang (Roswell Parker Cancer Institute, USA)

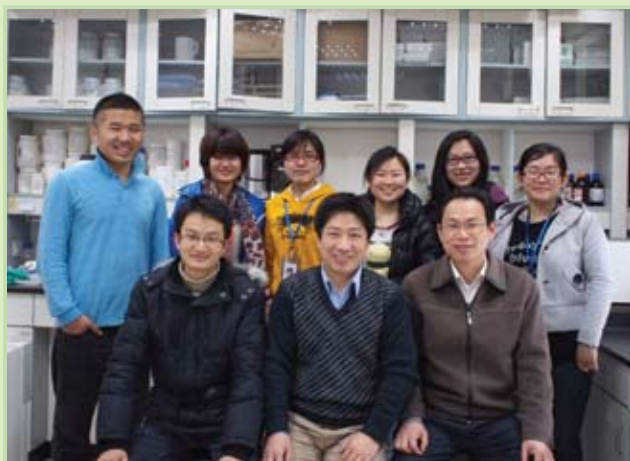
Presentation at the invitation at national and international conferences

Chinese Society for Microbiology, Hui Xiao, Heng yang, Nov.12

“The key role of Toll like receptors in inflammation and host defense”

2010 Publications

Xiao H., Yin W., Khan M., Gulen M.F., Zhou H., Sham H.P., Jacobson K., Vallance B. A. and Li X. (2010) Loss of single immunoglobulin interleukin-1 receptor-related molecule leads to enhanced colonic polyposis in Apc^{min} mice. *Gastroenterology*. 139, 574-85.



Gulen MF, Kang Z, Bulek K, Youzhong W, Chen Y, Sass Bak-Jensen K, Do JS, **Xiao H**, Delgoffe GM, Powell JD, Tuohy VK, Cua DJ, Li X. (2010) The receptor SIGIRR suppresses Th17 cell proliferation via inhibition of the interleukin-1 receptor pathway and mTOR kinase activation. *Immunity*. 32, 54-66.

Unit of Innate Immunity Research (Established in 2009)

Principal Investigator

Guangxun Meng

Ph.D, Immunology, Technical University of Munich, Germany. (2001-2005)

Post.Doc. Mucosal Immunity, National Institute of Allergy and Infectious Diseases (NIAID), NIH, USA. (2005-2009)

Team Members

Post-doctoral fellow: Liming Mao

Research Assistants: Zhao Wu, Hua Li, Jinling Dang, Weiying Fang

M.S-Ph.D students: Mingkuan Chen, Hongbin Wang, Wei Chen, Xiaomin Yao, Yue Xing

M.S. students (Co-supervising): Guowei Lei (Shandong Normal University)

Trainee: Cuili Dong, Yudan Chi

Research direction

NOD like receptors (NLR) are a recently discovered family of innate immune receptors involved in microbial pattern recognition and host inflammatory responses. A pyrin domain containing NLR, namely NLRP3, forms an inflammasome upon activation by a wide spectrum of microbial products as well as sterile challenge. The inflammasome converts procaspase-1 into its active form, the later process the proIL-1 β and proIL-18 into mature IL-1 β and IL-18 respectively.

Mutations in the NLRP3 gene are associated with a number of autoinflammatory diseases due to excessive IL-1 β production. Study with a “Knock-in” mouse model carrying an autoinflammation associated NLRP3 mutation revealed that the hyper-activation of the NLRP3 inflammasome not only results in over production of IL-1 β , but also leads to augmented Th17 cell differentiation, the latter amplifies inflammatory pathogenesis. Besides IL-1 β , however, other unknown factors from antigen presenting cells seem to be also contributing to the Th17 cell differentiation. One aim of this lab in the near future is to understand the detailed molecular mechanism of NLRP3 activation, and to identify those unknown molecules for further understanding of the in vivo connection between the NLRP3 inflammasome activation and Th17 cell differentiation.

In addition, certain viral infection induces IL-1 β and IL-18 production from antigen presenting cells through activation of NLRP3 and caspase-1. Applying NLRP3, ASC (Apoptosis-associated Speck-like protein with a Caspase-recruitment domain) and caspase-1 “knock-out” animals, inflammasome activation by Sendai and Influenza viruses has been studied and revealed interesting but controversial information. Our lab is going to explore viral activation of NLRP3 inflammasome in macrophages or dendritic cells by applying different viruses such as SARS, HBV, HCV, HIV, et.al., which should gain further insight into the understanding of viral activation of inflammasome and help to understand strategies from the host to cope with specific viral infection. The long-term aim of this study is to develop approach to control viral infection via regulation of inflammasome activation.

Research progress

1. The study of molecular mechanism of NLRP3 inflammasome activation

NLRP3 (NALP3, Cryopyrin, CIAS1 or PYPAF1), one of the most studied NOD-like receptors, has been linked to a large number of infections and autoimmune/autoinflammatory diseases. Characterized by a C-terminal leucine-rich repeat (LRR), a central NACHT domain and an N-terminal pyrin domain, the only known role of NLRP3 so far is to form a multi-protein complex termed NLRP3 inflammasome. The main function of inflammasome is to activate caspase-1, which then processes two important inflammation mediators, pro-IL-1 β and pro-IL-18 to their mature forms. To date, the detailed mechanism of NLRP3 inflammasome activation is still elusive. Originally, NLRP3 was thought to bind PAMPs directly like TLRs. However, as more and more heterogeneous PAMPs and DAMPs found to activate the NLRP3 inflammasome, it is becoming a consensus that these activators may trigger a common factor or event that leads to NLRP3 activation. Now we are studying the molecular mechanism of NLRP3 inflammasome activation in different aspects. The aim of this study is to develop approach to control autoinflammatory diseases via regulation of inflammasome activation.

2. Function of inflammasome in response to HCV infection

The NLRP3 inflammasome has been found to be involved in response to influenza virus infection. To dissect the possible role of inflammasome in HCV infection, we analyzed monocytes for inflammasome activation upon HCV challenge to figure out whether human primary monocytes and monocytes cell line THP-1 produce interleukin-1 β when exposed to HCV, and next we will study whether this process activates inflammasome. The related mechanism will be also investigated.

Research Support

- CAS 100-Talent Program (2009.12-2012.12)
“Function of NLRP3 inflammasome in inflammation and viral infectious disease”
- Grant for Excellent scientist from the Ministry of Human Resources and Social Security of China (2011.10-2013.9)
“Function of NLRP3 inflammasome in the development of colitis”
- Grant for Inflammation key project from NSFC (2011.1-2013.12)
“Mechanism study of NLRP3 in the development of colitis and colitis associated cancer”
- Shanghai Natural Science Foundation (2011.4-2014.3)
“Activation Mechanism Study of NLRP3 inflammasome.”
- Novo Nordisk-CAS Research Foundation (NN-CAS-2010-5 (SIBS))

Collaboration

Collaborations within IPS:

Jin Zhong, Bing Sun, Yan Zhang, Qibin Leng, Zhong Huang, Ke Lan

Others Collaborators:

Paride abliz (Xinjiang Medical University)

Lei Xiao (Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, CAS)

Guiwen yang (Shandong Normal University)

Future Plan

In the coming year our group will fulfill the following aspects of work:

- Build up a team of clarified personal duties, cooperative and coordinative spirits and positive attitudes;
- Publish 2-3 pieces of paper independently or in cooperation;
- Apply for several grants or funds;
- Carry out different cooperative research work within or beyond our institut;
- Participate in the construction work of different IPS' platforms;
- Positively involve in the IPS' employment, job evaluation, student enrollment, academic exchanges, project evaluation and education&training programmes.

Publications

Chen M, Wang H, Chen W and **Meng G***. Regulation of Adaptive Immunity by the NLRP3 Inflammasome. **Int.Immunopharmacol.**, 2010, Nov 27. [Epub ahead of print] (*Correspondent author)

Hu Y, Mao K, Zeng Y, Chen S, Tao Z, Yang C, Sun S, Wu X, **Meng G**, Sun B. Tripartite-Motif Protein 30 Negatively Regulates NLR Family, Pyrin Domain-Containing 3 Inflammasome Activation by Modulating Reactive Oxygen Species Production. **J Immunol.** 2010 Nov 3. [Epub ahead of print]



Unit of Anti-infection Immunity and Vaccine Research (Established in 2010)

Principal Investigator

Dongming Zhou

Ph.D in Pathology Biology from Xiangya School of Medicine, Central South University, China, (2001)

Postdoctoral fellow at University of Tennessee, USA (2002)

Postdoctoral fellow at University of Pennsylvania, USA (2002-2004)

Postdoctoral fellow at The Wistar Institute, USA (2004-2006)

Staff scientist at The Wistar Institute, USA (2006-2010)

Team Members

Research assistant: Jinyan Luo, Caihong Zhu, Zhendong Wang, Miao Ding

Research Objectives

Created in Sept. 2010, the Unit of Anti-infection immunity and vaccine research has two main objectives:

- Generate various chimpanzee adenoviruses as vaccine vectors, and develop novel vaccines to some certain infectious diseases based on recombinant adenoviral vectors.
- Study the fundamental mechanisms of host immune responses to infection.

Highlights of Achievements and Progress

1. Research projects on vector development and vaccine research

Adenoviral vectors originally derived from Chimpanzee are the most promising vectors in vaccine research. We already expanded and purified several wide-type chimpanzee adenoviruses and isolated their genomic DNAs, and now are working on the construction of these adenoviruses as vaccine vectors. Based on one chimpanzee adenoviral vector, termed AdC68, we generated several novel vaccines against influenza, rabies and *schistosomiasis*. We are going to test the immune responses and immune protection induced by these vaccines in mice firstly and then in relevant big animal models

2. Research projects on infection and host immunity

During the natural infection with influenza virus, people may get infected for several times at low dose in a confined space. In order to understand the immune responses involved in the natural influenza infection and to establish a more stringent challenging model for influenza vaccine research, we conducted a repeated low-dose influenza infection in mice, and explored the immune responses involved in this model. The results showed that repeated low-dose influenza infection caused more severe disease than single high-dose infection, while the single low-dose infection provided protection for a lethal challenge two months later. Virus specific T cell response in all infection groups were comparable except for the production of IFN- γ in 10 LD50 group was lower than that in other groups, suggesting T cell responses elicited in single low-dose groups might play roles in protection for the mice against a lethal challenge later, but T cell responses induced in repeated low-dose groups are not sufficient for providing protection against the repeated infection. T cell and B cell responses involved in this infection model will be further explored.

Grant

- Knowledge Innovation Program of the Chinese Academy of Sciences (2010.10-2013.9)

Collaboration

Intramural collaborators:

Ke Lan

Extramural collaborators:

Ertl HC (The Wistar Institute, Philadelphia, USA) -- Influenza A vaccine

Perspectives

- Continue to construct chimpanzee adenoviruses as vaccine vectors and develop novel vaccine vectors, and to test above novel vaccines in animals and explore the immune responses and immune protection induced by these vaccines. Initiate new projects with developing novel vaccines against other diseases including HPV vaccine.
 - Continue to explore the mechanisms involved in repeated low-dose influenza infection model.



Unit of Anti-infective research (established in 2010)

Principal Investigator

Ralf Altmeyer

Ph.D. Thesis on viral vaccine vectors, Institut Pasteur Paris, France 1991-1994

Post-Doc, Institut Pasteur Paris, France (Neuropathogenesis of HIV infections) 1995-1996

Team members

Laboratory secretary: Yimei Zheng

Ph.D students: Yize Li, Kai Wang, Jin Sun, Benjamin Bailly

Research assistants: Peijun Ren, Shuyang Tu, Xiaoxi Li, Shanshan Xu, E. Cydnie Bedford

Intern: Nevine El Khatib (Pediatric)

Research Objectives

Identification of novel therapeutic approaches striking at the virus-host interface. Using chemical probes and compounds in development and drugs for anti-infective therapy we aim to identify novel cellular targets and pathways leveraged by pathogens for efficient replication. Viral infections caused by Enterovirus 71 (Hand Foot and Mouth Disease), Adenoviruses (Viral epidemic keratoconjunctivities, severe infections of immunosuppressed transplant patients) and obligate intracellular bacteria Wolbachia (endosymbiont of nematodes causing Filariasis) are the main objectives of our lab.

Background

Obligate intracellular parasites require host cell mechanisms for efficient replication and can therefore be targeted for anti-infective therapy. Cellular targets have the advantage that they are not subject to high frequency of mutations which make them attractive as targets for pathogens with high mutation rates or genetically distinct subtypes. The example of the HIV receptor antagonist Maraviroc and the HCV treatment with type 1 Interferon illustrates that targeting the host can achieve antiviral activity *in vivo*.

Hand, foot and mouth disease (HFMD) has become as an emerging disease in China since March, 2008. It has struck nearly 3,4 million children of which over 30,000 were severe cases

with neurological complications. According to the Chinese Ministry of Health 1,355 children presenting with severe infections have died from the disease. Of note, severe cases and fatalities are rising dramatically with 126 deaths in 2008, 353 in 2009 (280% year-on-year increase) and 888 in 2010 (252% year-on-year increase; www.moh.gov.cn). HFMD is mainly caused by enterovirus 71 (EV71) and coxsackievirus A16 (CVA16), both members of the Picornaviridae family. EV71 tends to cause more severe disease and therefore has been paid more attention to than CVA16. EV71 infection usually causes only mild symptoms in the majority of patients and is mostly self-limited (Ref. 1). But a few patients with EV71 infection develop central neuronal disease manifesting as aseptic meningitis, encephalitis or poliomyelitis-like acute flaccid paralysis, and neurological pulmonary edema or hemorrhage. Pulmonary edema is the main cause of death in HFMD patients. So far, the mechanisms underlying the neurological pathogenesis of EV71 infection remain to be fully understood. Currently, there is no vaccine for immunoprophylaxis nor therapy to treat the disease. Drug development is hampered by the absence of a suitable animal model.

Adenoviruses cause a number of self-limiting but often highly infectious diseases that affect multiple organs, most commonly those associated with respiratory, genitourinary and gastrointestinal tracts and the ocular surface. Many factors have driven a search for effective topical and systemic antivirals to adenoviruses. These include patient morbidity, economic losses and chronic visual disturbances associated with epidemic keratoconjunctivitis; and the startling recent trend of high morbidity and rising mortality associated with systemic adenoviral infections in the immunosuppressed, particularly pediatric bone marrow transplant recipients. The development of effective antivirals has proven to be a complex task, owing to the fact that multiple and often genetically divergent adenovirus serotypes can cause similar diseases. Currently, there remains no licensed systemic or topical treatment. Ocular adenovirus infections are very common in Asia including China, Japan, Korea, Vietnam, Thailand and Cambodia. In Japan alone over 1 million cases are reported to a National Epidemiological Surveillance of Infectious Agents registry. Over half of the 51 adenovirus serotypes have been associated with ocular diseases. In an environment with a lack of effective antivirals, treatment is currently limited to symptomatic therapy and physician-recommended epidemiological control measures to reduce transmission; or to topical corticosteroid treatment to alleviate the immune infiltration. The latter can be difficult to manage by the physician, owing to rebound effects of these infiltrates following steroid withdrawal, and the adverse consequences of chronic topical steroid therapy (glaucoma, cataracts and microbial superinfection).

Natural Vitamin E components have recently been shown to display isomer-selective activity against Adenoviruses *in vitro* and *in vivo* in immunosuppressed Adenovirus-infected hamsters by our collaborators at Saint Louis University (R. Altmeyer, unpublished observations). However, the mechanism of action, breadth of action, optimal dose and ability of chemically modified

analogs to inhibit virus growth remains yet to be determined. Broad human exposure as nutritional and selected animal and human toxicity studies have shown that Vitamin E components have an excellent systemic safety record which supports their development for eye infections. We expect that this program will yield significant novel insight into the biology of Adenovirus infections and contribute to the development the first effective and safe drug against viral infections of the eye.

Obligate intracellular bacteria:

Filarial nematodes are important helminth parasites of the tropics and a leading cause of global disability. They include species responsible for onchocerciasis, lymphatic filariasis and dirofilariasis. A unique feature of these nematodes is their dependency upon a symbiotic intracellular bacterium, *Wolbachia*, which is essential for normal development and fertility. Antibiotic (anti-*Wolbachia*) therapy is a new treatment approach for human Filariasis. Initial trials using a six-week course of doxycycline treatment against *O. volvulus* were effective at depleting the bacteria and resulted in a block of embryogenesis of worms.

While clinical trial results argue clearly for the use of doxycycline for selected indications, the prolonged treatment regimen, evidence of resistance and the contra indication of Doxycycline for use in children under the age of 8 and pregnant women, require further development of anti-*Wolbachia* drugs.

Human parainfluenza viruses (hPIV) and the Respiratory Syncytial Virus (RSV) are both members of the *Paramyxoviridae* family, respectively from the *Paramyxovirinae* and *Pneumovirinae* subfamilies. Unlike their counterparts hPIV-2 and hPIV-4 that cause mild croup, hPIV-3 and hPVI-1 are currently the second major cause of respiratory disease in children after RSV. They mainly cause upper and lower respiratory tract infection often leading to bronchiolitis or pneumonia, and hospitalization. RSV, on the other hand, is the first cause of acute lower respiratory tract infections in newborns worldwide. The fact that only 3 to 5 % of the infected children are admitted to the hospital during the peak season shows the seriousness of the acute morbidity from RSV, also leading to severe pneumonia. Nowadays, 90% to 100% of the children older than 5 years of age possess antibodies against these viruses. As a matter of fact, they infect the majority of up-to-2-years old infants mainly during spring and early-summer months each year. The viruses being very contagious, it emphasizes the need for antiviral therapies as they have been found to be very infectious not only to children, but also to elderly and immunocompromized. Indeed, hPIV-3 for instance has been found to show symptoms similar to those caused by the H1N1 virus. Up till now, no drug or vaccine against any of them has been commercialized.

Grants

- Bill & Melinda Gates Foundation

- LKSF “LI KA SHING FOUNDATION”
- CAS Innovation funding
- SISEA “Surveillance and Investigation of Epidemics in Southeast Asia”

Collaboration

Intramural collaborators:

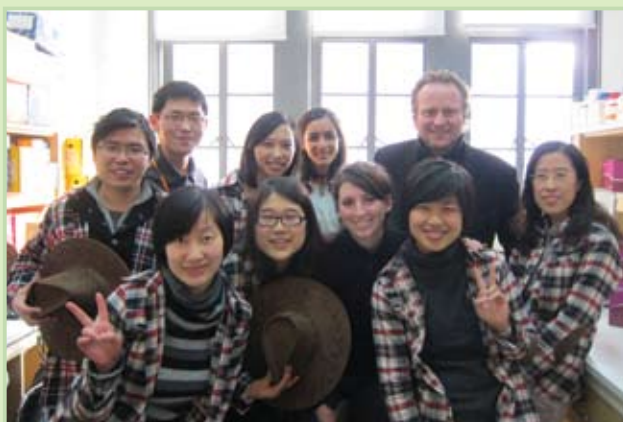
Bin Sun, Bin Li, Qibing Leng, Yan Zhang

Extramural collaborators:

- Mei Zeng (Children’s Hospital of Fudan University Qing Xie)-EV71
- Griffith University-hPIV&RSV

Publication

Wang K, Wang W, Yan H, Ren P, Zhang J, Shen J, Deubel V. Correlation between bocavirus infection and humoral response, and co-infection with other respiratory viruses in children with acute respiratory infection. *J Clin Virol.* 2010 Feb; 47(2):148-55.



Education

Graduate Course: Basic Virology and Viral Immunology was given to the first year graduate students of the institute, 11 lecturers involved into the program.

Date	Content	Lecturer
3/2, 2010	Viral replication – RNA viruses	Tetsuya Toyoda
3/2, 2010	Viral replication – retroviruses and DNA viruses	Tetsuya Toyoda
3/9, 2010	Virus structure and taxonomy	Rong Chen
3/9, 2010	Viral entry and un-coating	Rong Chen
3/16, 2010	History of virus research	Vincent Deubel
3/16, 2010	Biosafety in virology laboratory	Vincent Deubel
3/23, 2010	Viral assembly and egress	Rong Chen
3/23, 2010	Virus-host cell interaction	Jin Zhong
3/30, 2010	Viral pathogenesis	Paul Zhou
3/30, 2010	Cell transformation by viruses	Paul Zhou
4/6, 2010	Escaping from immune system by viruses	Zhong Huang
4/6, 2010	Vaccines against viral diseases	Zhong Huang
4/13, 2010	Basic virology techniques	Tetsuya Toyoda
4/13, 2010	Anti-viral drug therapy	Tetsuya Toyoda
4/20, 2010	Innate immune responses to viruses	Qibin Leng
4/20, 2010	Adaptive immune responses to viruses	Qibin Leng
4/27, 2010	Viral epidemiology	Vincent Deubel
4/27, 2010	Virus diagnosis	Vincent Deubel
5/4, 2010	Picornaviridae	Jin Zhong
5/4, 2010	Reoviridae	Jin Zhong
5/11, 2010	Flaviridae: JEV, DV, YFV, WNV	Jin Zhong
5/11, 2010	Hepatitis C virus	Jin Zhong
5/23, 2010	Rhabdoviridae and Filoviridae	Jianhua Wang
5/23, 2010	Coronaviridae	Bing Sun
5/25, 2010	Paramyxoviridae	Tetsuya Toyoda
5/25, 2010	Orthomyxoviridae	Tetsuya Toyoda
6/1, 2010	Polymaviridae and Papillomaviridae	Yan Zhang
6/1, 2010	Adenoviridae	Yan Zhang
6/8, 2010	Herpesviridae I: HSV, EBV, CMV	Ke Lan
6/8, 2010	Herpesviridae II: VZV, HHV6, HHV7, and KSHV	Ke Lan
6/12, 2010	Poxiviridae	Ke Lan
6/12, 2010	Hepadnaviridae and unclassified agents: HBV,	Ke Lan
6/22, 2010	Retroviridae: HTLV-1, HTLV-2 and HIV	Jianhua Wang
6/22, 2010	Viral vectors for gene delivery	Paul Zhou
6/29, 2010	Examination	

Student Journal club and seminar

In 2010, there were 36 presentations, PALL Life Science continued its contribution to this activity. 12 students received awards.

First semester:

Journal club:

1st grade award: Jie Lu (Jin Zhong's group)

2nd grade award: Deguang Liang (Ke Lan's group), Weiming Wang (Paul Zhou's group)

Seminar

1st grade award: Zhenguo Wen (Paul Zhou's group)

2nd grade award: Hongbing Jiang (T. Toyoda's group), Xianzhi Lin (Ke Lan's group)

Second semester:

Journal club:

1st grade award: Xiaohua Ye (Zhong Huang's group)

2nd grade award: Mingkuan Chen (Guangxun Meng's group), Xiaoxi Lin (Rong Chen's group)

Seminar

1st grade award: Jing Zhang (Bin Li's group)

2nd grade award: Hongbing Jiang (T. Toyoda's group), Ying He (Jin Zhong's group)



Awardees with Prof. Ralf Altmeyer, Director General, Prof. Bing Sun, Co-Director, Prof. Jin Zhong, chair of IPS Education Committee, representative from PALL Life Science.

Awards obtained by IPS professor and students

Prof. Paul Zhou

CAS Zhu-Li Yuehua Award for excellent Supervisor

Leiyun Weng (Ph.D student of Viral Genome Regulation Unit)

CAS Zhu-Li Yuehua Award for excellent Ph.D student

Cheguo Cai (Ph.D student of Anti-Viral Immunity and Genetic Therapy Unit)

CAS Di-Ao Fellowship Award (2nd grade)

Zhiheng He (Ph.D student of Tumor Virology Unit)

SIBS Pfizer Fellowship Award (Special Award)

Wanyi Tao (Ph.D student of Viral Hepatitis Unit)

SIBS Pfizer Fellowship Award (1st grade)

Kai Wang (Ph.D student of Emerging Viruses Unit)

SIBS Pfizer Fellowship Award (2nd grade)

Graduated students in 2010

Cheguo Cai, Anti-Viral Immunity and Genetic Therapy Unit, graduated with Ph.D academic degree

Shijian Zhang, Viral Genome Regulation Unit, graduated with Ph.D academic degree



Left: Shijian Zhang,
right: Cheguo Cai,
middle: Prof. Bing
Sun, Co-Director

Collaboration on graduate student education with University

On June 17, IPS-CAS and Suzhou University signed an agreement on education collaboration, to set up a M.S.-Ph.D program student under co-supervision system. On Sept.1, the first patch of 3 students from Suzhou University arrived IPS-CAS.

Training

4th PASTEUR-AREVA Course on Anti-Viral Immunity

The 4th PASTEUR - AREVA Course on Anti-Viral Immunity was organized by IPS-CAS on 17-21 May 2011. 16 scientists from USA, France, Germany, Japan and China gave lectures at the course. The topics cover innate immunity, acquired immunity, immune response to pathogen infection and vaccine. 186 students and researchers from nationwide universities and research institutes participated in the course, in which 10 university undergraduate students were supported by IPS-CAS. The value of the lectures and dynamic interaction during the course was highly appreciated by both lecturers and participants.

Students participation in international conferences and collaboration

On 2010, IPS-CAS encouraged and supported 20 students to participate international courses, conferences and collaboration in Hong Kong, Japan, Germany, France.

From May 6 to November 30, Mr. Yize Li of Emerging Viruses Unit stayed in the Laboratory of Viral Genome and Vaccination of Institut Pasteur in Paris for 7 months, working on a JEV project.

From July 2 to August 1, Mrs. Jin Sun of Emerging Viruses Unit stayed in the Lab of Virology Unit of Institut Pasteur De Cambodia for 1 month, working on serological study.



From July 6 to 24, 2 students participated in the 7th Virology Course in Hong Kong, organized by Hong Kong University-Pasteur Research Center.

From Aug.29 to Sept. 1, Mr. Deguang Liang, Mr. Xianzhi Lin of Tumor Virology Unit participated in the 13th Symposium on Kaposi virus and related diseases in UCLA, USA. They gave an oral presentation and poster presentation respectively.

From Sept.1 to Dec.1, Mrs. Zuojia Chen of Molecular immunology went to Muenster University in Germany for an internship of 3-month period, with support from NSFC of China.

From Sept.2 to 7, 4 students participated in the 7th Global Influenza Conference in Hong Kong.

From Sept 3 to 7, Mr. Shijian Zhang and Mr. Qiang Wang of Viral Genome Regulation Unit participated in the 7th Conference “Prevention and Control to Influenza” in Hong Kong. They presented their research by posters.

From Sept.10-14, 4 students participated in the 17th International HCV and Related Viruses Conference in Yokohama, Japan.

From Nov.3 to Dec.31, Mr. Baosen Jia of Tumor Virology spent two months in Institut Pasteur in Paris for collaboration research.

From Nov.21 to 24, 2 students participated in the annual meeting of International Network of Pasteur Institutes in Hong Kong. They presented their research progress by posters.



Academic exchanges

Guest seminars and lectures at IPS-CAS in 2010

Date	Lecturer	Affiliation	Topic	Host
February 4	Daniel Kolakofsky	University of Geneva Medical School	RIG-I does it all; but what exactly does it sense?	Ralf Altmeyer
March 15	Xu-yu Zhou	Institute of Microbiology, Chinese Academy of Sciences	Treg and Immune Regulation	Yan Zhang
March 24	Dongyang LI	Brookhaven National Laboratory	Mycobacterium tuberculosis proteasome and its selective inhibitors	Bing Sun
April 9	ROY CURTISS III	Arizona State University	New Technologies in Using Recombinant Attenuated Salmonella Vaccine Vectors	Zhong Huang
April 20	Taishan Hu	Oklahoma Medical Research Foundation	The role of c-Myb in NKT cell development	Bing Sun
May 21	Barry T Rouse	University of Tennessee	Tinkering with Regulatory Mechanisms to Change the Outcome of Virus Infection	Bin Li
May 28	Gang Dong	Vienna Bio-center	Structural Insights into the MHC Class I Peptide Loading Complex	Jin Zhong
May 31	Pierre J. Talbot	University of Quebec	Coronaviruses: from common colds to neurological disease and the lessons of SARS	Ralf Altmeyer
June 22	Yi Yang	East China University of Science and Technology	Imaging and Functional Studies of Redox Events and Thiol Proteome: unveiling the role of protein oxidation in biological processes	Bin Li
June 24	Mark von Itzstein	Griffith University	Viruses, carbohydrate recognition and drug discovery	Ralf Altmeyer
June 30	Yang-Xin Fu	The University of Chicago	The role of innate protection against acute infection in gut tissues	Bin Li
July 1	Zhiqiang Zhang	The University of Texas M. D. Anderson Cancer Center	Helicases pair with TRIF to sense dsRNA in Dendritic cells	Jin Zhong
July 2	Pinghui Feng	University of Texas Southwestern Medical Center	A model gamma herpesvirus activates the MAVS-IKKbeta pathway to promote viral lytic replication	Ke Lan

July 9	Hua Gu	Columbia University	Intracellular regulation of immunity and immune system malignancy	Bin Li
July 16	Rachel Caspi	National Eye Institute	Understanding autoimmunity to the eye through animal models	Guangxun Meng
July 19	Yasmine Belkaid	NIAID, NIH	Regulatory T cell in infectious disease	Bin Li
July 21	Luis Graca	Universidade de Lisboa	Regulation with unconventional regulatory cells: the contribution of Natural Killer T cells to immune tolerance	Bin Li
July 23	Yun-Cai Liu	La Jolla Institute for Allergy and Immunology	Protein Ubiquitination in Immune Regulation	Bin Li
Aug 2	Ning Wang	China CDC	Epidemiological characteristic of HCV serum in Chinese different group	Bin Li
Aug 19	Karin Loser	University of Münster	TNF family members in the regulation of cutaneous immunity	Bin Li
Aug 20	Peter Honkanen	Aushon Biosystems	Detection of biomarkers—High throughput multiple samples detection, screening method and service	Bin Li/ Ke Lan
Sept 1	Yi Ni	University of Heidelberg	How HBV orchestrates its infectivity determinants to execute the virus entry	Qiang Deng
Sept 14	Hans-Joachim Hoeltke	Roche	The latest application of Roche Diagnosis in infectious diseases	Ke Lan
Sept 14	Chuhu Yang	Roche	GS Junior: New generation gene sequencing system in life sciences	Ke Lan
Sept 20	Lydia M Sorokin	University of Münster	Vascular basement membranes as selective barriers to leukocyte infiltration into inflamed tissues	Bin Li
Sept 27	Stephan Ludwig	University of Münster	Targeting cell signalling pathways to fight the flu - towards a paradigm change in anti-influenza therapy	Bin Li
Oct 11	Zhiping Wu	Cleveland Clinic Foundation	Mass spectrometry analysis of lipid oxidation, a diagnostic tool for inflammatory diseases	Hui Xiao
Oct 12	Linsen Yang	Applied Biosystems	Attune acoustic focusing cell analysis and latest development	Ruihong Zhu
Oct 14	Fengyi Wan	Johns Hopkins University	Novel subunit of NF- κ B conferred regulatory specificity during infection by foodborne pathogen	Bin Li
Oct 18	Alan Sher	NIAID	Role of IL-1R signalling in innate immunity to Mycobacterium tuberculosis and in the adjuvant effects	Hui Xiao

Oct 18	Tianyi Wang	University of Pittsburgh	Curing HCV infection by treating its receptor addition: Fact or Fiction?	Jin Zhong
Oct 19	Eric Cohen	IRCM	Recent Advances in HIV-1 accessory protein Biology	Bing Sun
Oct 19	Zhiming Zheng	NCI	MicroRNA, cancer and tumor virus infections	Ke Lan
Oct 22	Laurent Rénia	A*STAR	Sterile protection against malaria induced by vaccination with live parasites	Ralf Altmeyer
Nov 9	Baochi Liu	Fudan University	Perioperative Therapy in Patients with HIV Infection	Jianhua Wang
Nov 10	Ken Ishii	National Institute of Biomedical Innovation	Innate control of vaccine immunogenicity by nucleic acid adjuvants	Zhong Huang
Nov 16	Tongqing Zhou	NIAID/NIH	From Neutralizing Antibodies to an Effective HIV-1 Vaccine: Structure-Based Approaches	Paul Zhou
Dec 6	Ming Tan	Cincinnati Children's Hospital Medical Center	Structural Basis of Norovirus-Host Interaction and Implications in Disease Control and Prevention	Rong Chen
Dec 20	Xiangping Qu	Tufts University	Export factors are needed for release of the mRNA 3'end processing complex after polyadenylation	Jin Zhong

Partnership

Partnerships with Chinese institutions

Joint laboratory for Vaccine Development with Hualan Biological Engineering Inc

On 27 April 2010, IPS-CAS and Hualan Biological Engineering Inc. signed a cooperation agreement on the creation of a Joint Laboratory for Vaccine Research and Development. The Joint Laboratory is set up at IPS-CAS and will focus on research for novel vaccines.

The Joint Laboratory is funded by both IPS and Hualan and receives support from the Chinese Academy of Sciences (CAS) with a goal to meet the country's strategic needs for new vaccines and to efficiently translate basic research innovation into products.

Prof. SUN Bing, head of the joint laboratory and co-director of IPS: "IPS is at the forefront of innovation in vaccine research in China. With Hualan we have found the right partner to develop our science to the pre-clinical and clinical stage".

Mr. AN Kang, the Chairman of Hualan Biological Engineering Inc., "The Hualan and Institut Pasteur of Shanghai compose a strong partnership which is engaged in new vaccine research and production. It will improve our vaccine research and development capability and technology level, speed commercialization to bring benefit to populations at risk.

Prof. Ralf Altmeyer, Director General of IPS: "The joint laboratory with Hualan is an example of the institute's new strategy in translational research. IPS aims to become the partners of choice for partnerships in Infectious Disease research and product development in China."

Investigation of severe and lethal cases of Hand Foot and Mouth Disease in children with Children's Hospital of Fudan University (CHFU)

In 2010, IPS-CAS signed a clinical research agreement with the Children's Hospital of Fudan University (CHFU) on hand foot mouth disease (HFMD), China's second most lethal infectious disease in 2010.

Since 2008, over 30,000 severe HFMD cases have been diagnosed in China and the disease has already cost the life of more than 1200 children*. Severe cases of HFMD are caused by Enterovirus 71 (EV71) which can infect the brain to cause encephalitis, poliomyelitis-like syndrome and/or pulmonary edema. There is no vaccine or drug against EV71 and a prognostic test for early detection of children developing severe complications is urgently needed. The Ministry of

Health considers HFMD drug and vaccine R&D a top public health priority.

The clinical research study is set for three years and is supported by grants from the Li Ka-Shing Foundation (Hong Kong), the French Agency for Development (Paris) and the Chinese Academy of Sciences.

The clinical and laboratory investigation by the joint team from IPS and CHFU will identify viral, immunological and host genetic factors responsible for severe infections in children. The study is open to other clinical research centers in China and the Asian region.

Collaboration on HCV with Hospital of Jilin Univ

With the support of National Science and Technology Key Projects on Major Infectious Diseases during the "11th Five Plan", IPS-CAS and No.1 Hospital of Jilin University set up a joint project on HCV clinical research. A treatment protocol for parts of patients from Fuyu town, Jilin was established. The objective is to find out the decisive factor involved in HCV therapy and body clearance, from the data collected from patients in this region, which are related to their natural history of hepatitis C virus infection as well as virological responses to the interferon therapy.

Activities of the Joint laboratory for Immunity and Infection with No.1 Hospital affiliated to Xinjiang Medical University

During 2010, two workshops were held between researchers of the joint laboratory. It was decided that the Joint Laboratory would set up a "Seed Fund", to support joint the projects. Currently, there are two on-going projects. One is "pathogenesis of KSHV's sarcoma" under the direction of Prof. Ke Lan of IPS and Prof. Hao Wen of the Hospital. Another project "mechanism investigation on inflammasome in Children fungal infection in Xinjiang" is to be implemented soon.

Internaitonal partnership

IPS-CAS continued the strategic partnerships initiated in 2005-2009 with AREVA, TOTAL, AIR LIQUIDE and Li Ka Shing Foundations



AREVA, LI KA SHING, TOTAL and AIR LIQUIDE foundations are essential partners of IPS-CAS. Thanks to their continuous support that includes scientific fellowships, research materials and financing of public health / awareness activities, IPS-CAS has been able to develop ground-breaking activities in the fight against China's most prominent infectious diseases.

AREVA	TOTAL	LI KA SHING	AIR LIQUIDE
<p>AREVA Foundation has been supporting IPS-CAS since 2005 on the issue of climate change and its impact on viral diseases.</p> <p>AREVA is associated with one research team at IPS-CAS on HIV/AIDS research. A scientific seminar on AIDS also brought together researchers, physicians and biologists to discuss state-of-the-art technological advances. The partnership also includes AIDS training designed to raise awareness about the disease among AREVA personnel in China.</p>	<p>Since 2008, TOTAL is associated with two research teams at IPS on Hepatitis B and C. The donation supports fundamental, vaccine- and therapy- oriented research on both viruses, as well as awareness actions directed towards doctors and medical students on transmissible diseases, including hepatitis but also STD. The partnership also includes HepB/HepC training designed to raise awareness about the disease among TOTAL personnel in China.</p>	<p>The donation, established in 2005, supports the collaborative program between IPS-CAS and the Joint Influenza Research Center of Hong Kong University on health programs combating avian influenza and other respiratory diseases.</p> <p>Priority of the collaboration is given to rapid and specific detection of known or novel agents and to the understanding of the interaction of viruses with the innate immunity of the host.</p>	<p>AIR LIQUIDE foundation has been a partner of IPS-CAS since 2009. The AIR LIQUIDE project aims at establishing more rapid and efficient medical diagnostics in the treatment of respiratory infections. The diagnostic units use "biological microchips", a new molecular biology technology.</p> <p>Thanks to Air Liquide contribution, IPS-CAS hopes to discover many viruses and develop more efficient diagnostic methods to improve the treatment of these illnesses.</p>

Visuals from AREVA forum, picture of Xiaowei delivering speech, one picture of researchers, one picture of Pathogen Diagnostic Center

IPS-CAS initiated new international partnerships in 2010

The following new partnerships with foundations, institutions and academic organizations were initiated:



GRIFFITH UNIVERSITY	LSTM / SIMM
<p>Creation of a collaborative research facility titled “Australia-China Joint Drug Discovery Laboratories”:</p> <p>Establishment of joint laboratories drawing together the complementary infectious disease expertise of IPS-CAS and the drug discovery facilities and medicinal chemistry expertise of Griffith University</p> <p>Focus on anti-infective/viral drug research and early stage development; development of Industry partnerships through early stage technology development and late stage technology licensing</p> <p>Exchange of students and academic staff members; delivery of seminars, conferences and workshops</p>	<p>IPS-CAS acts as Project Manager and High Throughput Screening Center for the Liverpool School of Tropical Medicine (LSTM), involved in the fight against tropical diseases.</p> <p>LSTM aims at screening Shanghai Institute of Materia Medica (SIMM)’s Traditional Chinese Medicine compounds in an in vitro infected cell line with material provided by SIMM. Successful compounds will then be tested in vivo at IPS animal facilities.</p>

In line with its development plans towards industrial collaborations, IPS-CAS also established relationships with new partners in the pharmaceutical and biotech industry:



PALL LIFE SCIENCE	NOVO NORDISK	CHRONOS	FILLIGENT
PALL Life Science supports IPS-CAS student activities: Journal Club, Student Seminars, various events.	NOVO NORDISK provides scholarships to outstanding young scientists at IPS-CAS.	IPS conducts a study on in vivo activity of a CHRONOS THERAPEUTICS compound on the ovariectomized mouse model of osteoporosis. The output of this study will determine whether said compound is effective at reducing loss of bone mineral density.	IPS-CAS will perform in vitro screening of FILLIGENT's anti-infective compounds against viruses. The project involves first-pass read screening of novel solubilised compounds in cell-based assays for anti-viral activity.

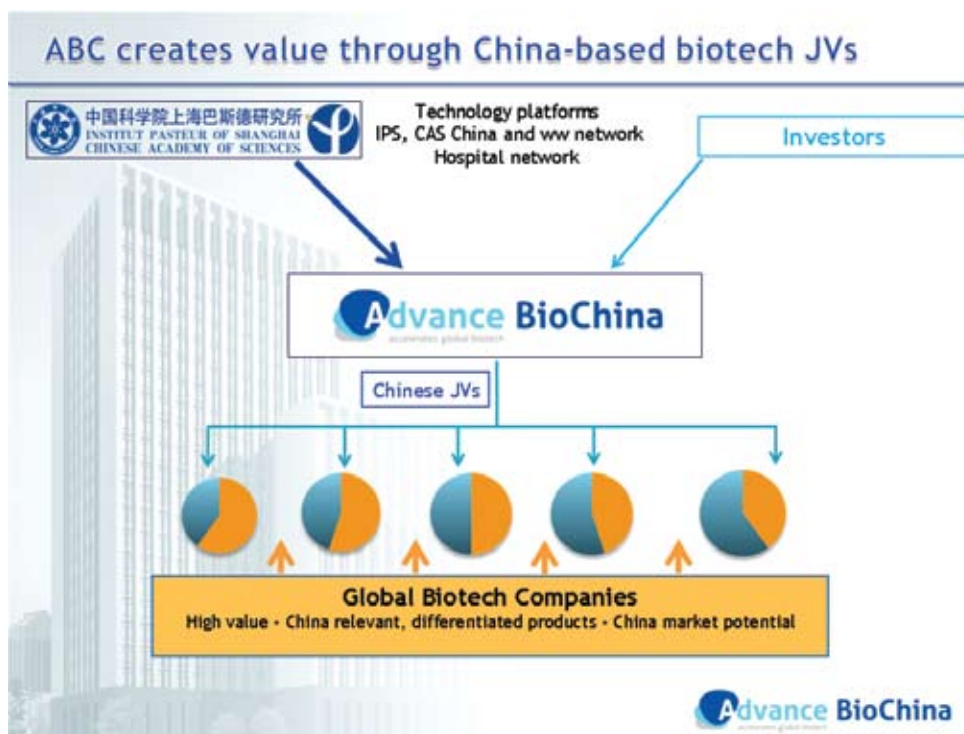
IPS-CAS officially started its global biotech incubator / accelerator “Advance BioChina”, a proactive, broad business development initiative towards global and local actors of the biotech and pharmaceutical industry, to accelerate and facilitate their China market access

Based on the scientific strength of the Institute, its integration within national and international networks, its full-fledged infrastructure and highest level scientists, and in line with China’s strategic priorities for the 2010-2020 decade giving priority to application-oriented research, IPS-CAS has set a new ambition in developing and intensifying its partnerships with biotech and pharmaceutical companies: Advance BioChina.



Advance BioChina is an R&D-focused, for-profit subsidiary of IPS-CAS, and positions itself as the gateway to China for global biotech companies. Advance BioChina co-invests with global biotech companies into Shanghai-based Joint Ventures.

Advance BioChina will incubate and develop up to 25 companies over the next 5 years to help them develop innovative products in the field of vaccines, therapeutics and diagnostics, for the Chinese and global markets. Advance BioChina selects projects initiated by foreign or Chinese biotech companies with a strong China relevance. Through the creation of Joint Ventures, Advance BioChina provides scientific/clinical co-development as well as China market access acceleration services to its partners. Thus biotech companies will benefit from IPS-CAS’ experience in China, its scientific expertise and technology platforms to achieve their product development goals.



China's pharma market has grown 15-20% p.a. over the last 8 years and is forecast to become the world's second largest biggest pharma market by 2020. The healthcare and IP-law reforms in China are improving the R&D environment and commercial perspectives for differentiated medical products. In that context, Advance BioChina customers will benefit from a plug-and-play China establishment platform and fully leverage Chinese scientific resources, including potential financial support (for early stage up to pre-clinical proof of concept projects) and regulatory fast track (for later stage projects involving clinical trials).

ABC accompanies Biotech Companies through critical development steps in China

- Specific segment of the value chain: pre-clinical/clinical data to clinical PoC
- Reach major value inflection points through high quality and lower cost R&D
- Develop up to 25 companies in China over 5 years (3-5 years incubation)



Besides providing access to funding and full incubation services, Advance BioChina will constantly pay special attention to secure the intellectual property of its JV partners.