Combining Immunotherapy and Vaccination to Treat Chronic Infections

FONDATION MERIEUX
MEETING REPORT- V3

The Combining Immunotherapy meeting was organized by Fondation Mérieux held in Les Pensières Conference Center in Veyrier du Lac – France, June 2009.

The following report summarizes the information provided during this meeting based on abstracts and speakers’ lectures; procedure specifics for the research investigation are not detailed in this report.
Meeting reporter: Valentina Picot, DVM
Edited by Farshad Guirakhoo, PhD
Disclaimer
Information on this report was obtained from the lectures and abstracts given by the speakers as per scientific agenda at the ‘Combining Immunotheraphy’ Fondation Mérieux meeting held in June 2009 at “Les Pensieres” conference center in Veyrier du lac, France. All graphs, flow charts and images were obtained from the speakers’ presentations to facilitate the comprehension on the subject.

The information in this report was authorized as per signed authorization form by the speakers in question, a modified form of dissemination of this information might require further speakers’ authorization.
The information provided does not constitute a manual or technical sheet on the subject; it might have omissions; we cannot assure its completeness or accuracy; it should not be used for the diagnosis or treatment of disease. Commercial products and prototypes are named and illustrated for information purposes only, no endorsement or recommendation by the Fondation Mérieux, the organization partner or the meeting reporter are implied or should be inferred. They do not necessarily represent the views of Fondation Mérieux or that of the organization partner or the meeting reporter and have not been formally disseminated and should not be construed to represent any agency determination or policy.
Contents

I. Introduction
II. Summary of Presentations and Discussions

Keynote lecture:
Genetically determined susceptibility to infections with M. Tuberculosis: Lesson for the development of immunotherapeutic strategies

Session 1:
Basic Information on Immunology and Physiopathology of Infectious Diseases

Session 2:
Infections and Chronic Degenerative Diseases

Session 3:
Immunotherapy Approach

Session 4:
Vaccination and Combination Approaches
**Introduction**

In chronic infections, one or more infectious agents, mostly viruses or bacteria, replicate in a host despite its immune response, sometimes cause life-long diseases. After a host has been invaded by the pathogen, many criteria determine victory or defeat for the host. Generally, victory against the pathogen requires balanced response from the host; too much response can damage the host itself, whereas too little response allows the pathogen to reside in the host and establish a “chronic” infection.

In an acute infection, immune T-cells recognize the antigens of the pathogen, expand dramatically in numbers, and become effector T-cells. These effector T-cells, usually in millions, search for the antigens and hereby kill the pathogens. They also produce cytokines such as interferon and TNF molecules, which can help clear infections. Since the high number of effector T-cells cannot be sustained for a long time, most of these cells die off, leaving a subset remaining as memory cells which can be reactivated if the pathogen returns in the future (natural or vaccine-induced immunity).

In case of chronic infections, however, these text-book scenarios are not usually followed. The pathogen can evade the host immune system and establish chronic infection, possibly due to exhaustion of effector T-cells encountering high antigen load from the pathogen. A number of viruses and bacteria have, in fact, been associated with chronic diseases. For example, AIDS, caused by the Human Immunodeficiency Virus (HIV), is set to join heart disease and stroke as the top three causes of death world wide. It has killed more than 25 million people since it was first recognized on December 1, 1981, and it is estimated that at least 117 million people will die from the disease by 2030. Similarly, Tuberculosis (TB), caused by a bacterium called Mycobacterium tuberculosis, has sent about 200 million people to the grave over the past hundred years, and kills 1.6 million people every year world-wide despite an existing vaccine. These 2 diseases also make a deadly combination, TB-HIV, each accelerating the other’s development. In Africa, HIV is the single highest driver of the number of TB cases; 70% of all new active TB cases occur in HIV infected individuals. Chronic Hepatitis B Virus (HBV) is another preventable chronic infection, affecting about 350 million people worldwide, and causing 650,000 premature deaths annually. Hepatitis C Virus (HCV) infects another 130 million people and causes 250,000 deaths annually despite the availability of effective tools for immunotherapy.

In addition to HBV and HCV, other sexually transmitted, chronic diseases such as Human Papilloma Virus (HPV; causing cervical intraepithelial neoplasia) and Herpes Simplex Virus (HSV; causing blisters affecting genitals as well as the eyes or skin) are major contributors to the number of global chronic infectious diseases which plague the population.

Epstein-Barr virus (EBV), which causes another very common chronic viral infection, is not only the cause of mononucleosis in up to 50% of infected individuals but is also implicated in conditions such as Burkett’s lymphoma, nasopharyngeal carcinoma, chronic fatigue syndrome, and multiple sclerosis, to name a few.

Among bacterial and fungal pathogens, H. pylori, Mycoplasma, Chlamydia, and Listeria are widely spread and cause millions of chronic infections world-wide. Clinical manifestations associated with Chlamydia pneumoniae infection continue to emerge beyond respiratory illness. After much controversy, recently compiled evidence was gathered by a diverse methodology including molecular biology, immunohistology, serology, animal model, and ultra structure. These results hint to a casual evidence of association of Chlamydia pneumoniae with majority subsets of multiple sclerosis and Alzheimer disease. These new findings may have major implications in treatment and prevention of these diseases.
Current products and future need:
According to the World Health Organization, no-one dies from old age anymore. All deaths are attributed to diseases, 65% of which are preventable. Despite great advancement in the field of vaccination and immunotherapy, only a few vaccines (e.g. for HBV and HPV) are available against these major chronic diseases. One reason might be the high genetic variability (e.g. for HCV and HIV and/or molecular mimicries (H. Pylori) of these pathogens. Nevertheless, it seems that prophylactic vaccines against HSV-1 and HSV-2 will soon be available, which will ensure the reduction of new infections and, in long term, help to reduce the burden of the disease if used on a global scale. In the meantime, immunotherapy approaches using peptides, subunit proteins, cytokines and immunomodulators, DNA immunization, RNAi, VLP, viral vectors, live attenuated viruses or antibodies, should be useful in suppressing the viral load and reducing the rate of spread of infections among the population at risk.

Farshad Guirakhoo, Ph.D.
Farshad.guirakhoo@sanofipasteur.com
**Keynote lecture**
Genetically determined susceptibility to infections with M. Tuberculosis: Lesson for the development of immunotherapeutic strategies

**Human genetics of mycobacterial infections: Lesson for the development of immunotherapeutic strategies**
*Laurent Abel, INSERM, France*

Although rarely pathogenic, poorly virulent mycobacteria, including live BCG vaccine and most environmental mycobacteria (EM), may cause a variety of clinical diseases, while *Mycobacterium tuberculosis* and *M. leprae* are more virulent, causing tuberculosis (TB) and leprosy, respectively.

Remarkably, only a minority of individuals develop clinical diseases, even if infected with virulent mycobacteria. The interindividual variability of clinical outcome is thought to result to a large extent from variability in the human genes that control host-response to mycobacterial infection.

**TB: Individual variability in clinical outcomes**

In this presentation, different methods and strategies that can be used to identify human genes controlling infectious diseases, especially those concerning simple and complex inheritance of predisposition to mycobacterial diseases in humans were reviewed.

Methods of investigation in humans

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Rare (disseminated forms)</th>
<th>Common (TB, Leprosy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causality</td>
<td>Monogenic</td>
<td>complex</td>
</tr>
<tr>
<td>Tools</td>
<td>Mendelian Genetics</td>
<td>Genetic Epidemiology</td>
</tr>
<tr>
<td>Sample</td>
<td>Small</td>
<td>Large</td>
</tr>
</tbody>
</table>

Rare mutation  
Strong effect

Common polymorphism  
Modest effect
Mendelian susceptibility to mycobacterial diseases (MSMD): rare patients with disorders, IL-12/23-IFNγ pathway, have been found to be vulnerable to BCG and EM. This is a very rare but often due to genetic disease (consanguinity).

MSMD: 6 genes, 13 genetic diseases

Complete IL12R-β1 deficiency: No cellular response to IL-12 results in Mendelian tuberculosis.
There is a complex predisposition to common mycobacterial diseases and a very large spectrum of clinical manifestations.

**Tuberculosis**  
*(M. tuberculosis)*

M. tuberculosis: Approximately 8 million new cases per year; 90% of infected subjects do not develop the disease.

M. *Leprae*: Approximately 400,000 new cases per year; 95% of infected subjects do not develop the disease.

Substantial advances have been made recently in the genetic dissection of leprosy by identifying two major genes, *PARK2/PACRG* and LTA, by positional cloning. These two genes are strongly associated with the risk of developing leprosy *per se* (all clinical subtypes). In the case of TB, the first major locus was identified recently by genome-wide linkage screen, which was mapped to chromosome 8q12-q13 in a Moroccan population consisting of 96 multiplex families with a total of 227 offspring (92% >15 years, 90% < 40 years) with positive pulmonary TB.

**Linkage to chromosome 8q12-q13**

![Linkage plot](image)

39 families with affected parent  
All 96 families  
Consistent with dominant inheritance  
Linkage disequilibrium mapping is ongoing
While precise identification of this major gene is ongoing, the fascinating observation of these last years is the demonstration that TB can also reflect a Mendelian predisposition. As mentioned before following the findings obtained in MSMD, several children with genetic IL-12/23-FNy circuit, in particular those with complete IL12RB1 deficiency were found to have severe TB as their sole phenotype. The proportion of children with disseminated TB due to Mendelian predisposition remains to be experimentally determined, but has been estimated at 3% to 30% by Bayesian statistics.

Overall, these recent findings provide the proof of concept that human genetics of mycobacterial infections involve a continuous spectrum from Mendelian to complex predisposition with intermediate major gene situations.

Genetic predisposition to mycobacterial infections $\rightarrow$ continuous spectrum

- **Mendelian mutations with causal role demonstrated**
  - direct clinical and therapeutic implications (disseminated TB of children)
  - information on immunological pathways ($\rightarrow$ candidate genes)

- **Intermediate major gene effects**
  - in specific populations, phenotypes, age class (LTA and leprosy) …
  - implications $\sim$ Mendelian

- **Common polymorphisms with moderate effect**
  - molecular basis difficult to validate (same pathway, interest of GWAS)
  - may have strong attributable risk at the population level
The understanding of the molecular genetic basis of TB will have fundamental immunological and medical implications, and will serve vaccine development in at least three ways. First and foremost, understanding the genetically determined immunological deficits that cause a small minority of children and adults to develop TB will allow designing vaccines that can bypass, boost or compensate for these deficits.

For example, if primary or reactivation TB develops because of insufficient production of a specific, immunologically related cytokine, this will indicate which pathway should be targeted by the candidate vaccine, while providing the most relevant correlates of protective immunity to be tested in order to assess vaccine efficacy.

Secondly, this genetic information will make it possible to test candidate vaccines in at-risk individuals only. Individuals naturally resistant to TB represent a major confounding factor in vaccine trials. Thirdly, the genetic dissection of TB will facilitate the detection of the related primary immunodeficiencies which potentially predispose to live vaccine-caused diseases and deaths.

Overall, understanding the immunological deficits that cause a minority of children and adults to develop TB will not only facilitate the rational design of vaccines that can overcome these deficits but also pave the way to the development of new treatments based on the restoration of a deficient immunity (e.g. treatment by IFN gamma in children who have an impaired production of IFN gamma due to a primary IL12RB1 deficiency).
Session 1: Basic Information on Immunology and Physiopathology of Infectious Diseases

Immunomodulation during Herpesvirus Latency
Erik Barton, Purdue University, USA

Herpes is a very diverse group of viruses, consisting of about 18 Herpes viruses broken down into Alpha, Beta and Gamma:

- **Alpha**:
  - HSV1: recurrent cold sores
  - HSV2: recurrent genital sores
  - VZV: chicken pox and shingles
- **Beta**:
  - CMV: severe birth defects
  - HHV6 & HHV7: rash in newborns
- **Gamma**:
  - EBV: mononucleosis, lymphoma
  - KSHV: Kaposi’s sarcoma

The above are the major common diseases caused by these viruses, but it is important to bear in mind that Herpes viruses adapt incredibly easy to the human host. All humans are infected with multiple Herpes viruses during early childhood. After clearance of acute infection, Herpes viruses enter a dormant state known as latency. Latency persists for the life of the host and is generally asymptomatic, but in the context of immune compromise Herpes viruses can reactivate to cause recurrent disease.

The interaction of Herpes virus and host has a characteristic 3 phase infection cycle, with different disease scenarios depending upon host-pathogen interaction:

![Stages of Infection with Herpesviruses](image)

If Herpes infections are acquired early in life, as most people do, the first infection is mild and generally does not show the severity that infection and disease acquired as adolescent or adult do.

The period of viral replication is followed by a phase of latency characterized by the absence of detectable high levels of viral infection and essentially no symptoms. This latent period can last for decades, and because of the lack of viral presence, the immune response is basically on resting memory cells. Historically, it was thought that the latency phase was completely dormant with regards to immune response; however, recent new technology showed that this
is not the case, neither for the host immune response nor for the virus, as shown in the following graphs.

Evidence in both human and animal Herpes virus systems indicates that virus-antigen-specific lymphocytes persist at high levels during latency, and often express markers of recent antigen exposure. This suggests that latency is restrained by frequent, low level immune activation. We speculate that this active immune surveillance might have by-stander consequences for the host response against self or environmental antigens.

As observed, there is chronic lymphocyte activation during the latent infection phase resulting in low activation of memory cells. The consequence of this in the host is that reactivation attempts are rapidly detected and inhibited before reactivation reaches a high level.

The surveillance role of these T and B cells is important to maintain the virus from reactivating; however, when a host is immune compromised, the surveillance is voided and virus reactivation is high causing severe disease.
The study presented here focuses on this latent phase of the Herpes virus cycle knowing that chronic lymphocyte activation is occurring at low level; viral gene expression and soluble inflammatory mediators are also produced at low level.

The question that rises is: what are the consequences for the host response to other antigens?

There are a number of models stating that this is detrimental for the human host and others that it is beneficial, as shown below:

**Immune Modulation During Herpesvirus Latency**

Two models were reviewed here:

- “Cross-protection” from heterologous infections
- Enhanced immune surveillance of pre-cancerous cells
- Prevention of allergy or autoimmunity: the “hygiene hypothesis”

Using either murine gammaherpesvirus 68 or murine cytomegalovirus, which is genetically highly similar to the human pathogens Epstein-Barr virus and human cytomegalovirus, respectively, we demonstrated that latently infected mice are resistant to infection with the bacterial pathogens Listeria monocytogenes and Yersinia pestis.
All control mice did not survive.

- 1st week results: Active virus infection does not alter resistance to bacterial infection.
- 4th week: Striking results if waiting 4 weeks: latent virus infection renders the host resistant to bacterial infection.
- 12th weeks: The protective effect wanes, but is still significant at 3 months post-infection.

Latent infection but not acute infection has a profound effect on the secondary response to a bacterial challenge.

It was also determined that latency-induced protection is not antigen specific but involves prolonged production of the antiviral cytokine interferon-gamma and systemic activation of macrophages.
Differences in cytokine expression:
Latent MHV68 Infection is Characterized by Elevated Serum Levels of IFNg and TNFa

![Graph showing cytokine levels](image)

These cytokines are known to:
- Be released by activated lymphocytes
- Control Herpes virus replication non-cytolytically
- Activate phagocytes during bacterial infection

Furthermore, mice latently infected with murine gammaherpesvirus 68 are profoundly resistant to transfer of congenic tumor lines, and this resistance is mediated by prolonged latency-induced activation of host natural killer (NK) cells. Thus, whereas the immune evasion capabilities and lifelong persistence of Herpes viruses are commonly viewed as solely pathogenic, our data suggest that latency is a symbiotic relationship that provides benefits for the immune competent host.

Summary: Enhanced Innate Immunity During Herpes Virus Latency
- Latent b- and g-herpesvirus infection in mice confers striking cross-protection from lethal bacterial infection.
  - requires latent infection, lasts ~six months
  - is associated with systemic macrophage activation
  - is functional against diverse bacterial, viral, and protozoan pathogens
- MHV68 latency promotes arming of NK cells in vivo.
  - associated with increased survival in a lymphoma transfer model
  - may explain the difference between NK cell function in humans and pathogen-free mice
  - role for Herpes virus in resistance to tumorigenesis?
Significance
- Herpes virus latency in mice upregulates the setpoint of innate immunity
- Herpes virus latency may represent a form of co-evolved, mutualistic symbiosis
  - universal vaccination against Herpes viruses may alter this balance and deprive the
    host immune system of critical regulatory signals.
- The “normal” human immune system is shaped by chronic viral infections that do not cause
  clinically evident disease and are absent in pathogen-free mice.
  - There are no Herpes virus-free humans
  - A comprehensive understanding of human immunology may require animal models
    infected with these viral symbionts.

Human HBV and HCV Infections
Thomas Baumert, University of Strasbourg, France

First, an overview of the global impact of chronic Hepatitis B and C virus infection:
- 350 and 170 million HBV and HCV infected individuals
- Major cause of chronic hepatitis world-wide
- Leading cause of liver cirrhosis and hepatocellular carcinoma
- Major indication for liver transplant
- Effective and safe vaccine for HBV but not for HCV
- Antiviral therapy limited by resistance and high costs

HBV – Hepatitis B Virus

HBV: Viral Life Cycle

HBV: Clinical Course

Mother-to-child transmission: 90% chronic infection
Person-to-person transmission: 95% clearance

Mechanisms of cytotoxic T-lymphocyte (CTL)-induced liver disease and viral clearance
As presented in the following graph, there are two paths, the fas ligand/perforin pathway or the cytokines pathway, leading to cytotoxic or non-cytotoxic outcomes.
In the last decade, there has been a tremendous progress in the development of antiviral treatments. The following graph shows that treatment of HBeAg-positive chronic hepatitis B patients controls HBV infection but has limited viral clearance.

Another issue is the antiviral resistance after therapy for chronic hepatitis B, which represents another challenge for the development of new antiviral drugs.

HCV – Hepatitis C Virus

Clinical Course of Hepatitis C Virus Infection
There are similarities but important differences between the clinical course of HBV and HCV. In HCV infection, the clinical course is usually asymptomatic, and only a small fraction of infected individuals clear the virus and, thus, through the years the development of liver cirrhosis and hepatocellular carcinoma.

A breakthrough in the understanding of these viruses is the development of tissue cell culture models that allows the study of HCV infection.

There are models as:

- Full-length HCV-JFH1 (Japanese Fulminant Hepatitis 1)
  > Highly efficient replication in transfected cell lines

- Generation of cell culture-derived infectious HCV-JFH1 virions (HCVcc)
  > Envelope-dependent infection of naïve Huh-7 cells

Within these models, the virus cycle is observed.
As well as to determine the HCV Infection adaptive host immune responses as cellular immune responses though studies have also shown non cellular immune responses on the activation of neutralizing antibodies.
Mechanisms of HCV clearance

- Viral clearance - rapid induction of vigorous and broad HCV-specific CD4 and CD8 T-cell responses and virus-neutralizing antibodies
- Viral persistence – delayed, inefficient or absent cellular responses and delayed onset of virus-neutralizing antibodies with viral escape

The role of neutralizing antibodies is shown in the following graph for cleared and chronic Hepatitis C, respectively, from a study performed in 2007:
A woman with cleared disease shows an earlier onset of neutralizing antibodies than a patient with chronic disease.

$p = 0.0007$

$p = 0.0021$

$p = 0.011$

$p = 0.001$
Mechanisms of viral evasion: HCV re-infection during liver transplant
End-stage hepatitis C virus-related cirrhosis and hepatocellular carcinoma are a major indication for liver transplant, as characterized by:

- Universal re-infection of the graft
- Absent strategy for prevention of re-infection
- Accelerated progression of disease to cirrhosis
- Low efficacy and poor tolerance of antiviral therapy

Enhanced viral entry and escape from neutralization mediate viral evasion during re-infection of the liver graft

Selection of Viral Variants Immediately after Liver Transplant (Days 0-7)
- Enhanced infectivity of variants
- Escape from neutralizing antibodies

Prevention of HCV Re-infection in Liver Transplant
- Defined HCV variants are selected following transplant
- Selected variants demonstrated
- Highest infectivity compared to non-selected variants
- Less efficient neutralization than non-selected variants
- Viral entry and escape from neutralization - key determinants for the selection of variants
- Inhibition of HCV entry by monoclonal cross-neutralizing antibodies promising strategy for prevention of HCV re-infection of the graft

In summary, we have demonstrated that selected variants are characterized by higher infectivity than non-selected variants and are less efficiently neutralized than non-selected variants. These data suggest that viral entry and escape from antibody-mediated neutralization represent important determinants for the selection of variants during re-infection. Viral entry is a promising target for antiviral strategies preventing HCV re-infection of the graft including monoclonal antibodies and entry inhibitors.
Interferon-alfa based antiviral therapies – progress and challenges

- Marked progress in the treatment of chronic hepatitis C during the last decade: pegylated interferon-alfa and Ribavirin for 24 or 48 weeks
- IFN-based treatment limited by viral resistance, marked toxicity and high costs (€ 15,000 for 48 week combination therapy)

Novel Antiviral Treatment Approaches in Clinical Trials

Hepatitis B and C virus infection: progress and challenges

Marked progress:
- Established and emerging antiviral strategies for prevention (HBV) and treatment (HBV and HCV)
- Novel model systems for infection have allowed a better understanding of virus-host interactions and pathophysiology

Grand challenges:
- Mechanism of viral resistance and evasion (HBV, HCV)
- More efficient and better tolerated treatment strategies aiming at viral clearance (HBV, HCV)
- Prevention of HCV infection (vaccine, strategies for liver transplant)

Immune Dysregulation in HIV infections
Claire Chougnet, Cincinnati Children’s Hospital Research Foundation, USA

Chronic progressive HIV infection is usually associated with weak HIV-specific T-cell responses. A variety of lines of evidence have suggested that regulatory T-cells (T-reg), CD4+ T-cells, play an important role in ineffective immune control of viral replication in chronic progressive disease. First, in vitro T-reg depletion increases anti-HIV T-cell responses in HIV-infected individuals, something seen with SIV infection of macaques as well. Further, chronic progressive HIV disease and SIV disease are associated with increased numbers of T-reg (defined CD4+ FOXP3+ cells) in lymphoid tissues, compared with either non-progressors or uninfected individuals. Finally, in both humans and macaques, tissue FOXP3 levels are highly correlated with HIV/SIV viral loads during infection.

Interestingly, exposure of T-reg to HIV selectively promotes their survival via CD4-qp120 dependant pathway. On the other hand, our recent data in in vitro systems indicate that T-reg can suppress HIV production by effector T-cells. While the in vivo relevance of this remains to be defined, these data suggest that T-reg may well be double-edged swords in HIV infection: providing some protection to the host by limiting viral replication, at the cost of impairing immune responses of the virus itself —thereby facilitating the development of chronic progressive infection.

Protection against Chlamydia trachomatis infections
Luis de la Maza, University of California, USA

Chlamydia trachomatis is the most prevalent sexually transmitted bacterial pathogen present in the Southern part of the world, such as India, Africa, or South America, with an estimated 100 million diagnosed cases occurring annually. It is also the most common cause of preventable blindness; an estimated 150 million people suffer from active trachoma; 6 million are blind as an indirect result of Chlamydia infection.

Chlamydia trachomatis is the most prevalent sexually transmitted bacterial pathogen present in the Southern part of the world, such as India, Africa, or South America, with an estimated 100 million diagnosed cases occurring annually. It is also the most common cause of preventable blindness; an estimated 150 million people suffer from active trachoma; 6 million are blind as an indirect result of Chlamydia infection.

<table>
<thead>
<tr>
<th>Serovars</th>
<th>Site of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - C</td>
<td>Eye (trachoma)</td>
</tr>
<tr>
<td>D- K</td>
<td>Genital</td>
</tr>
<tr>
<td>LGV1, 2, 3</td>
<td>Lymphogranuloma venereum</td>
</tr>
<tr>
<td>C. muridarum</td>
<td>Mouse pneumonitis (MoPn)</td>
</tr>
</tbody>
</table>
These acute manifestations generally resolve over a period of a few weeks. However, in certain patients, long term sequelae may develop, including pelvic inflammatory disease, ectopic pregnancy and infertility. Furthermore, in areas of the world with poor hygienic conditions, *C. trachomatis* causes chronic infections that result in trachoma and lymphogranuloma venereum. Although effective antibiotic therapy is available, eradication of this pathogen will most likely only be achieved through a vaccination program.

**C. trachomatis clinical manifestations**

Men: urethritis, epididymitis, proctitis, Reiter’s syndrome  
Women: cervicitis, urethritis, salpingitis, infertility, ectopic pregnancy, chronic abdominal pain.  
Newborns: conjunctivitis, pneumonia, prematurity.

The most common clinical presentations are urethritis and cervicitis.

Men and woman can infect each other, and women infect newborns at the time of birth. *C. trachomatis* is the most common cause of pneumonia in infants up to 6 months.

The WHO has established an anti-trachoma program called SAFE in the hope to eliminate this disease by the year 2020. 
S= surgery, A= antibiotics, F= face wash, E= environmental changes,  
However, the main way to eliminate this disease is by taking care of “E” environmental and sanitary conditions in the affected regions and preventable disease.

Decades ago, in an attempt to prevent trachoma, vaccine trials with viable or inactivated whole organisms were performed.
- 1960-1970: Trachoma (humans and monkeys; guinea pigs)  
- 1980-Present: Genital tract infections (mice; guinea pigs)

The conclusions drawn from those early trials were that the vaccines for trachoma had an effect in preventing the onset of trachoma; the protection, however, was of short duration, serovar specific, and in some of the vaccinated individuals that were re-exposed to *Chlamydia* and developed a severe disease, the hypersensitization was longer lasting than the protection. This hypersensitivity reaction was thought to be due to a component present in *Chlamydia*.

The fact that the protection was serovar specific led to the conclusion that the antigen involved in eliciting the immune response was unique to each serovar. Based on these observations, recent efforts have focused on developing a subunit vaccine using as antigen the *C. trachomatis* major outer membrane protein (MOMP), which is the antigen that is serovar specific.

**C. trachomatis major outer membrane protein (MOMP): advantages**

- Surface exposed to Chlamydia  
- Abundant in the outer membrane  
- Highly immunogenic: has B- and T-cell epitopes  
- A majority of patients infected with *C. trachomatis* develop antibodies to MOMP  
- Most likely antigen responsible for the observed protection in the trachoma vaccine trials (antigen specific)
MOMP constitutes 60% of the mass of the outer membrane, is surface exposed, and numerous T- and B-cell epitopes have been mapped in this protein. Furthermore, DNA sequencing of MOMP showed that this protein is serovar-specific.

Many of the prior trials to develop a MOMP vaccine failed; however, it was clear that the conclusions from the first trachoma trials were true in relation to the MOMP being serovar antigen specific. Thus, a new approach to formulate a MOMP vaccine to induce protection was established using:

- The native *C. trachomatis* MoPn MOMP as the antigen.
- Adjuvants that stimulate a Th1 response and can be utilized in humans.

**Criteria for *C. trachomatis* vaccine efficacy**

- **Microbiological**
  - Number of animals with positive cultures
  - Number of inclusion forming units (IFU)/group
  - Length of time animals have positive cultures
- **Functional**
  - Number of fertile animals
  - Number of embryos per pregnant mouse

An animal model was developed for the following experimental plan:

- **Mice:** 3-4 week old BALB/c mice
- **Antigen:** Native *C. trachomatis* MoPn MOMP (10 µg/mouse)
- **Controls:** positive: intranasal EB (104 IFU), negative: ovalbumin
- **Adjuvants:** Cpg-1826 + Montanide ISA 720
- **Route of immunization:** i.m. + s.c. (x 3)
- **Immune response:** humoral and CMI
- **Challenge:** 105 IFU *C. trachomatis* MoPn in the left ovarian bursa
- **Fertility:** the mice were mated twice starting at 6 weeks following the intrabursal challenge
- **Measures of protection:** vaginal cultures and fertility

Inoculation of the genital tract of female mice with *C. trachomatis* results in an infection that parallels what has been described in humans. Using this model, Pal et al. (Infect. Immun.73:8153,2005) showed that systemic immunization of mice with the native *C.
*trachomatis* MOMP (nMOMP), formulated with CpG and Montanide as adjuvants, elicited an immune response that protected against a subsequent genital challenge.

The vaccinated mice had a significant decrease in the severity and length of vaginal shedding in comparison with the control animals.

A subunit vaccine, formulated with native MOMP, is as effective as immunization with live EB as shown by:
- The number of animals with positive vaginal cultures.
- The decrease in the number of IFU recovered from the vaginal cultures.
- The length of time of vaginal shedding.
- The number of pregnant mice.
- The number of embryos per mouse.

Furthermore, the vaccinated animals were protected against infertility.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>% Fertile micea</th>
<th>Mean no. embryos (+1SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leftb</td>
</tr>
<tr>
<td>MOMP</td>
<td>75 (15/20)c</td>
<td>2.6±2.4d</td>
</tr>
<tr>
<td>EB</td>
<td>81 (17/21)c</td>
<td>2.6±1.5d</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>10 (2/20)</td>
<td>0.2±0.5</td>
</tr>
</tbody>
</table>

a. % Mice with embryos in both uterine horns
b. Mice were challenged in the left uterine horn
c. P<0.05 by the Fisher exact test
d. P<0.05 by the Mann-Whitney U test

Recently, Kari et al. (J. Immunol. in press) showed that this type of vaccine can partially protect against an ocular challenge. Cynomolgus monkeys (*Macaca fascicularis*) were immunized systemically with nMOMP and challenged ocularly. Immunized monkeys exhibited a significant decreased in infectious burden during the early peak shedding periods. However, at a later time, they exhibited no difference from control animals in either burden or duration of infection. Immunization had no effect on the progression of ocular disease. These results show that systemically administered nMOMP is highly immunogenic and elicits protection against genital and ocular *Chlamydia* challenges.

This type of vaccine may have a significant impact in the control of acute and chronic *C. Trachomatis* infections.

Ongoing and future work
Prepare a recombinant MOMP with antigenic characteristics similar to those of the native MOMP.
Search for additional protective antigens.
Assess the efficacy of other adjuvants and routes of immunization.
Explore the efficacy of the vaccine in a male mouse model.
Work with industry on the formulation of a vaccine for humans.
In Western countries, atherosclerosis is the leading cause of morbidity and mortality; both genetic and environmental factors play a role in the pathogenesis of the disease. Classical risk factors include hypercholesterolemia, arterial hypertension, smoking, obesity, and diabetes mellitus. However, recent studies and several data (C reactive protein, fibrinogen, and soluble adhesion molecules) suggest that atherosclerosis is a chronic inflammatory disease reflecting a condition of endothelial cell damage and dysfunction.

Endothelial cell (EC) injury is considered to be an initial event in the development of atherosclerosis (endothelial-leukocyte adhesion molecules) which may lead to EC apoptosis and atherosclerotic lesions.

There are many infections related to atherosclerosis, such as: Chlamydia pneumoniae, Helicobacter pylori, Mycobacteriae, or Cytomegalovirus.

Infectious agents seem to be involved in endothelium damage by inducing an autoimmune response to Heat Shock Proteins (HSP). Human Cytomegalovirus (hCMV) infection is a common infection in adults (seropositive 60-99% globally), and is associated with endothelial cell damage, with restenosis after angioplasty and, recently, with high arterial blood pressure, a classical risk factor for atherosclerosis.

Antibodies against HSP60 are present in the majority of patients with coronary artery disease, and their titre correlates with disease severity. We have previously shown that autoantibodies against HSP60 are present in most atherosclerotic patients, and that these autoantibodies are directed to the sequence at position 153-163 of HSP60 (AELKKQSKPVT).

This amino acid sequence of HSP60 shows homology with two hCMV-derived proteins, UL122, early expressed during infection, and US28, a C-C chemokine receptors-like, involved in the reactivation of the virus from latency. Antibodies directed against HSP60 153-163 cross-react with the viral proteins.

<table>
<thead>
<tr>
<th>HSP60 153-163</th>
<th>A</th>
<th>E</th>
<th>L</th>
<th>K</th>
<th>K</th>
<th>Q</th>
<th>S</th>
<th>K</th>
<th>P</th>
<th>V</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>UL122 152-162</td>
<td>G</td>
<td>P</td>
<td>R</td>
<td>K</td>
<td>K</td>
<td>S</td>
<td>K</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>HSP60 153-163</td>
<td>A</td>
<td>E</td>
<td>L</td>
<td>K</td>
<td>K</td>
<td>Q</td>
<td>S</td>
<td>K</td>
<td>P</td>
<td>V</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US28 26-36</td>
<td>T</td>
<td>D</td>
<td>V</td>
<td>L</td>
<td>N</td>
<td>Q</td>
<td>S</td>
<td>K</td>
<td>P</td>
<td>V</td>
<td>T</td>
</tr>
</tbody>
</table>

**HSP60, hCMV e Atherosclerosis**

Anti-US28 and anti-UL122 affinity purified antibodies obtained from atherosclerotic patients, purified against the HSP60153-163 peptide and against the two hCMV-derived peptides bound non-stressed endothelial cells upon interaction with cell surface molecules through a mechanism of molecular mimicry. Such antibodies induced apoptosis of endothelial cells.

Indeed both viral peptides, UL122 and US28, show sequence homology with molecules normally expressed on endothelial cell surface:
- US28 shows homology with integrin alpha 6 (CD49f)
- UL122 show homology with CD151 and with connexin 45

<table>
<thead>
<tr>
<th>Connexin 45</th>
<th>G</th>
<th>P</th>
<th>R</th>
<th>E</th>
<th>K</th>
<th>K</th>
<th>A</th>
<th>K</th>
<th>V</th>
<th>G</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>UL122</td>
<td>G</td>
<td>P</td>
<td>R</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>S</td>
<td>K</td>
<td>R</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td>CD151</td>
<td>Q</td>
<td>L</td>
<td>R</td>
<td>K</td>
<td>K</td>
<td>A</td>
<td>S</td>
<td>G</td>
<td>R</td>
<td>V</td>
<td>A</td>
</tr>
<tr>
<td>US28</td>
<td>T</td>
<td>D</td>
<td>V</td>
<td>L</td>
<td>N</td>
<td>Q</td>
<td>S</td>
<td>K</td>
<td>P</td>
<td>V</td>
<td>T</td>
</tr>
<tr>
<td>CD49f</td>
<td>F</td>
<td>R</td>
<td>V</td>
<td>I</td>
<td>N</td>
<td>L</td>
<td>G</td>
<td>K</td>
<td>P</td>
<td>L</td>
<td>T</td>
</tr>
</tbody>
</table>

We have shown that anti-US28 antibody translocation on the membrane and release in the supernatant of sHSP60. The sHSP60 released from endothelial cells is able to trigger signaling through TLR4. Therefore, TLR4 activation may be considered a bridge between innate and acquired immunity in the pathogenesis of atherosclerosis.


Showing that hCMV infection can be involved in the pathogenesis of atherosclerosis: the virus can induce an autoimmune response that leads to endothelium damage and apoptosis through a mechanism of molecular mimicry involving the engagement of molecules normally expressed on the cell surface.

The same (auto) antibodies cross-react with hHSP60, thus amplifying endothelial cell damage.

Also, the gene expression profiles in endothelial cells were analyzed using the Human Genome U133A GeneChip® (Affymetrix). The GeneChip® Human Genome U133A is a single array representing 14,500 well-characterized human genes. This was done by:

- Stimulating endothelial cells with antibodies purified against either the UL122 or the US28 peptide (test samples) or with antibodies purified against an irrelevant peptide (control samples) for 6 and 12 hours.
Analyzing the different gene expression patterns using the Array Assist software version 2.0 (Stratagene, La Jolla, California, United States).

Modulated Genes in Huvec’s

The huvec was stimulated with anti-US28 and anti-US122 antibodies. Gene expression analysis demonstrated that both subsets of anti-hCMV antibodies modulated genes encoding for molecules known to be involved in the inflammatory process and in the pathogenesis of atherosclerosis: adhesion molecules (ICAM-1, VCAM-1, Selectin E), Chemokines (MCP-1, MIP2a), molecules involved in cell activation (MAPK, NF-kB), extra-cellular matrix deposition and growth factors (PGF, EGFR).

Anti-US28 antibodies modulated a higher number of genes (907 up- and 1389 down-regulated) than anti-UL122 antibodies (186 up- and 140 down-modulated genes).

This observation suggested that a different mechanism could be involved in the endothelial damage induced by the two subsets of anti-hCMV antibodies.

Conclusions:
- Anti-US28 and anti-UL122 affinity purified antibodies induce apoptosis of non-stressed endothelial cells; anti-UL122 antibodies induce apoptosis earlier than anti-US28 antibodies do.
- Both anti-hCMV antibodies modulated genes encoding molecules known to be involved in the inflammatory process and in the pathogenesis of atherosclerosis:
  - Adhesion molecules (ICAM-1, VCAM-1, Selectin E)
  - Chemokines (MCP-1, MIP2a)
  - Molecules involved in cell activation (MAPK, NF-kB)
  - Growth factors (PGF, EGFR)
  - Extra-cellular matrix deposition
- Anti-US28 antibodies induce translocation on the membrane and release in the supernatant of sHSP60.
- The sHSP60 released from endothelial cells is able to trigger signalling through TLR4.
- TLR4 activation may be considered a bridge between innate and acquired immunity in the pathogenesis of atherosclerosis.

Finally, we have recently analyzed the serum concentration of anti-UL122 and US28 peptide antibodies in a series of patients with CAD, and have correlated the concentration of such antibodies with the severity of the atherosclerotic disease.

**Genetics of Alzheimer’s Disease and Ideas for Treatment**

Ekaterina Rogaeva, University of Toronto, Canada

The most common form of dementia is Alzheimer’s disease (AD), which has a complex etiology involving multiple environmental and genetic factors. Main events in the pathogenesis of AD are the accumulation of Aβ peptide, a neurotoxic proteolytic derivative of the amyloid precursor protein (APP); and the formation of intra-neuronal tau-associated neurofibrillary tangles.

A general cascade responsible for Alzheimer Disease

AD could be viewed as a chronic infection

- Bacterial infection: *Chlamydia pneumoniae* was found in brain areas affected by AD (host cells: astrocytes, microglia and neurons) [Balin at al, 1998]; However, this is still a very controversial view.

- “Infection” by a mis-folded protein: many new discoveries have been found about mis-folded proteins as possible “etiology” for AD, for instance:
  - The accumulation of Aβ activates events that also injures neurons:
    - misprocessing of tau
    - tau filaments could be infectious [Clavaguera, et al 2009]
    - microglial and astrocytic inflammation
  - Majority of amyloid plaques in AD patients are surrounded by auto-antibodies [Kellner, et al Ann Neurol. 2009];
  - Anti-amyloid therapies include Aβ immunization and small molecules that reduce Aβ toxicity.
About 5% of the cases have early-onset familial AD, as genetic analyses have proofed.

Shown above are the three causal genes and their mutations (as per a study): *APP*, *PSEN1* and *PSEN2*, responsible for the early onset of AD. The APOE gene is a well-replicated risk factor for the common form of AD with onset after 65 years of age.
FACTS: Mutations causing AD cause mis-processing of APP
HYPOTHESIS: novel AD genes also cause mis-processing of APP

All the above shown genes and mutations can be responsible for a genetic form of AD development.

Recently, we demonstrated that the genetic variations in $SORL1$ gene could lead to changes in the trafficking of APP through intracellular compartments.

$SORL1$ is a sorting switch, forcing APP into recycling pathways

When the APP molecule is working correctly, it is located in the recycling pathway and can go back to the membrane; however, when it is down regulated and SORL 1 is not functioning, the APP is forced into a late endosomal pathway producing the accumulation of amyloid. Defects in SORL1 cause APP to be trafficked into regions where $A\beta$ is produced.
SORL1 is reduced specifically in cortical neurons in late-onset AD

Conclusions of SORL1 study
- Several SORL1 variants are associated with AD in multiple datasets;
- SORL1 variants are associated with reduced levels of SORL1 leading to more Aβ peptide;
- Novel therapeutic strategy: enhance SORL1 level

Multiple avenues for anti-amyloid therapies based on APP Processing

• Preventing amyloid formation via
  – γ/β-secretase inhibition
  – α-secretase stimulation
  – slow-down Aβ aggregation
• Blocking Aβ’s harmful effects
• Removing Aβ from the brain by active Aβ immunization
  – Elan Pharmaceuticals trial I, 2000

What works to reduce pathology and improves behavior in AD mouse models?
Aβ immunization, NSAIDs (γ-secretase modulators), Cholesterol management, Inhibitors of
Aβ aggregation, Green tea, Nice cages, Marijuana, Nicotine …..

Lessons from Aβ Immunization Trial
• Clearance of Aβ-plaques do not prevent disease progression: other features of AD
  remain (e.g. tau accumulation);
• We need to better characterize neuron-damaging pathways initiated by Aβ to develop
  new therapies (genetics could provide key players);
• The correct use of anti-amyloid therapies could be prophylactic in people at risk (as
determined by genetic factors).

Altered APP/ Aβ metabolism is a unifying theme for all known genetic causes of AD. The
knowledge about AD genetics is opening the way for the development of effective therapies
for AD. This will entail the identification of individuals predisposed to AD before they are
affected while the neuronal damage is still negligible. The genetic knowledge of AD has
generated many key questions for future research. What early pathways are affected by toxic
Aβ peptides? Can these downstream pathways be monitored diagnostically and manipulated
therapeutically? Indeed, in order to protect the brain from neuronal loss, it is essential to
understand the downstream damage mechanisms leading to AD.
Atherosclerosis is a chronic inflammatory disease of the vasculature commonly leading to myocardial infarction and stroke and joins the major causes of morbidity in the world. Current treatment for the disease mainly tackles the disease end stages, some therapies include:

- Risk factor reduction (e.g. stop smoking)
- Surgical (angioplasty, bypass)
- Thrombosis prevention (e.g. warfarin)
- Lipid reduction (e.g. statins)

Atherosclerosis develops through a number of years; timeline goes from several decades on endothelia dysfunction, the formation of atheroma, fibrous plaque and complicated lesion and rupture.

The phase in which this study is interested in is the progression of the disease in 5% cases into the formation of an atherosclerotic plaque, complicated lesion and rupture where the mechanisms are poorly understood.

The stability of the atherosclerotic plaque depends on the burden of inflammation. The base of this stability is not yet well known, many different antigens have been proposed as the ox-LDL. A large burden of inflammation tends to lead to plaque instability.

Interestingly, it is observed that the size of a lesion makes no difference in a plaque being safe or unsafe, what matters is the structure of the plaque.

The problem for the cardiologist is that in an angiogram, two plaques of similar size, whether safe or unsafe, will look identical, thus making it difficult to determine which plaque will rupture and which will not.
The unstable plaque

Platelets aggregate at site of erosion

Activated MΦ accumulate lipid, induce intimal SMC death and degrade matrix in the fibrous cap

CD4+ T cells secrete Th1 cytokines which activate MΦ

Large free lipid core

Intimal SMCs become senescent and apoptotic

EC Leukocyte transmigration

Calcification

(RANK/OPG)

The above Figure shows some of the mechanisms that occur in unstable plaques. Thrombus can form and extend into the lumen; the plaque can rupture which, in most cases, results in death.

There seems to be an imbalance between cytokine production and plaque inflammation

Th2/Tregs

PROTECTIVE

IL-4
IL-6

Th1

PRO-ATHEROGENIC

IL-1β
IL-2
IL-12
IL-18
TNFα
IFNγ

Studies have shown that some cytokines seem to be protective and pro-repair of the plaque as IL-1ra, IL-5, IL-10 etc and other seem to work pro-inflammation as IL-1beta, IL-2, etc this through Th1 or Th2 pathways. Also, IL-33 seems to play a role in atherosclerosis stability; no other studies than the one presented in this talk have been performed regarding this cytokine and this disease.

IL-33 is:

- Novel IL-1 family member
- Discovered 2005 (Schmitz et al)
- Homology with IL-18
- Does NOT require caspase cleavage for activation
- Strong inducer of Th2 immune responses (independent of IL-4)

Stromal cells – fibroblasts, endothelial cells, macrophages seem to secrete IL-33; macrophages secrete only under special circumstances.


The AIM project was performed to assess the role of the IL-33/ST2 pathway in atherosclerosis showing that IL-33, novel IL-1-like cytokine signals via ST2, can reduce atherosclerosis development in ApoE -/- mice on a high fat diet. First, it was determined that IL-33 and ST2 are present in the normal and atherosclerotic vasculature of mice and humans.
Expression of ST2 and IL-33 mRNA in vascular cells and tissues, as the following graph A shows. IL-33 is highly expressed in mice on a HFD (high fat diet), and ST2 is evenly expressed in the different groups of mice.

Also, as shown above in example B, IL-33 and ST2 are also expressed in human vascular cells.

Then the experimental design was drawn and the study performed:

Male ApoE^{-/-}  
Commence high fat diet  
6  
12  
18  
WEEK (after birth)  

GROUP 1 – PBS or IL-33 (1ug)  
GROUP 2 – IgG-Fc control or sST2-Fc (50ug)  
GROUP 3 - IL33 (1ug) or IL-33 (1ug) plus αIL-5 (30ug)  

2x/week i.p. for 6 weeks  
End Cull mice

The study results showed that while control PBS-treated mice developed severe and inflamed atherosclerotic plaques in the aortic sinus, lesion development was profoundly reduced in IL-33 treated animals.

IL-33 reduced atherosclerosis in the aortic sinus of ApoE-/- mice.

IL-33 also markedly increased levels of IL-4, IL-5 and IL-13, but decreased levels of IFN gamma in serum and lymph node cells. IL-33 treatment also elevated levels of total serum IgA, IgE and IgG1, but decreased IgG 2a, consistent with a Th1-to-Th2 switch.

IL-33 treated mice also produced significantly elevated anti-ox-LDL antibodies. Conversely, mice treated with soluble ST2, a decoy receptor which neutralizes IL-33, developed significantly larger atherosclerotic plaques in the aortic sinus of the ApoE -/- mice compared to control IgG-treated mice. Furthermore, co-administration of an anti-IL-55 mAb with IL-33 prevented the reduction in plaque size and reduced the amount of ox-LDL antibodies induced by IL-33. In conclusion, IL-33 may play a protective role in the development of atherosclerosis via the induction of L-5 and ox-LDL antibodies.

IL-33 – potential therapeutic options
- IL-33 immunotherapy for CV disease unlikely – long time course of disease progression and side effects
- Possible use in acute conditions, eg MI with local delivery
- BUT sST2 may have potential use as a biomarker for patients to predict outcome post-MI? or as a surrogate for other trials?
- Inhibition of IL-33 e.g. with neutralising Abs may have use in Th2 diseases, e.g. asthma
Immunotherapy in HIV infection: Current and future challenges.
Yves Levy, INSERM CHU Henri Mondor, University Paris, France

Administration of HAART has resulted in significant improvements in the survival of HIV-infected patients. However, despite now reaching a point where we can achieve durable, maximal suppression of plasma viral load in most of our HAART-treated patients, non AIDS-related morbidity and mortality among these patients remain a concern. Conditions typical of aging, such as cardiovascular disease and cancer, are seen at a higher rate in HIV-infected patients compared to the general population, potentially because the ability of HAART to restore immunocompetence appears incomplete even in patients who have long-term undetectable HIV-1 RNA.

Studies show that long term suppressive HAART does not result in normalization of CD4 T-cell counts in the majority of individuals. Most HIV positive individuals under HAART treatment with complete viral suppression do not reach the 800 CD4 T-cell counts, which is considered the minimum count for normal individuals. Studies have shown that patients under HAART reach a point in time where there is no gain of CD4 T-cell count.

Following, some factors of the paradox of incomplete CD4+ T-cell count restoration despite successful c-ART:
Age; insufficient thymic output, reduced T-cell proliferation capacity and higher rates of T-cell apoptosis; insufficient suppression of HIV-1 RNA while on HAART; higher levels of collagen disposition/ fibrosis and activation-induced damage to lymphoid tissue architecture; among other factors.

The theoretical targets of immune-based therapeutic approaches to improve CD4 T-cell count in HIV patients under HAART include working on the thymic output to improve the generation of naive T-cells and in the peripheral expansion of CD4 T-cells. IL-7 and IL-2 are cytokines which have a similar mode of action and influence the peripheral CD4 T-cell expansion, but IL-7 also have an impact on the maturation of CD4 as well as CD8 T-cells.

It seems that cytokines IL-7 and IL-2 working as an adjunct to HAART therapy can help in the immune reconstitution in HIV infection, although studies show that each cytokine might have a different benefit impact on the CD4 T-cell reconstitution.

In a trial conducted in 2007, HIV patients who were given HAART therapy and IL-2 showed a sustained increased of CD4 T-cells. Even when, at the end of the trial, IL-2 was stopped, the CD4 T-cell counts did not drop in those patients. Based on these data, two phase III trials were performed in 6000 patients, the SILCAAT and the ESPRIT trials. In each trial, patients have initially 300 and 350 T-cell counts, respectively, and in a randomized manner were given HAART alone or HAART and cycles of IL-2, respectively, for one year and longer as needed at the drop of the CD4 T-cell count.

In each trial, patients indeed showed a raise in the CD4 T-cell count as compared to the controlled patient group, and in most individuals this raise was maintained over a long period even after IL-2 was stopped. However, the results were disappointing because despite a CD4 T-cell count raise, the incidence of OD and death compared to the control group was not significant. Thus, the conclusion from this ten-year trial is that IL-2 is a key factor for peripheral CD4 T-cell expansion; however, this CD4 T-cell count raise under IL-2 therapy is not associated with a better clinical outcome.

There are several hypotheses that try to explain why CD4 raises under IL-2 did not confer clinical benefits, such as: CD4 T-cells induced by IL-2 have no role in host defense as compared to c-ART expanded CD4 T-cells. The CD4 T-cells triggered by IL-2 are mostly naive and memory cells in comparison with ART, which are memory but also effector CD4 T-cells, thus each being qualitatively different. Another hypothesis is the phenotype of the CD4
T-cells under IL-2, as these cells express the CD25 antigen and are not functional in vivo, the potential benefits of IL-2 are counteracted by other negative effects of IL-2 in the long-term, as it was observed that the benefits of IL-2 in the patients treated were limited in the long term.

Another study showed that giving patients IL-2 prior to HAART interaction allowed these patients to maintain significantly higher levels of CD4 T-cell counts than the control group. It is important to highlight that there was no difference between the two groups in terms of CD4 T-cell count at 18 months.

In IL-2 treated patients, it is observed that mainly the CD4+CD25+ T cells persist and are still significant in the long term even in the absence of IL-2 therapy. This means that IL-2 completely changes the homeostatic regulation of these populations of CD4 T-cells, since almost 70% of the CD4 T-cells found in the blood are IL-2 expanded CD4 T-cells with CD25 marker.

Another study has been performed in patients under IL-7 cytokine therapy, a phase I trial that observed T-cell recovery in HIV-1 infected adults. Patients with CD4 T-cell counts below 400 where given IL-7; counts were different than those portrayed for IL-2. Cell count raise was dose dependant, and not only CD4 but also CD8 T-cell counts significantly increased. The increase of CD4 T-cells was in central and effector memory cells.

This is a very interesting cytokine; however, there is much more to discover in order to determine its safety and efficacy.

Another concern is how to target persistent viral replication; in patients treated with HAART there is an undetectable viral load, however, there is persistent viral replication. Thus, these individuals have reservoir of latent infected cells that the immune system cannot target because there is no expression in these cells of HIV antigens.

In order to target these reservoirs, there are different approaches to allow the expression of HIV in these cells; some studies have shown that IL-7 increases the expression of HIV antigens in the cells, the HIV replication. This suggests some strategies to treat patients with IL-7 to allow the expression of these infected cell reservoirs and then to target them with an antiviral therapeutic vaccine.

Thus, a next challenge is to try to combine IL-7 with therapeutic immunization, this aiming to: Develop vaccine strategies aimed at eliciting poly-epitopic immune responses to avoid escape mutations and to boost the quality of immune responses beyond that elicited by natural infection (use of cytokines, chemokines, costimulatory molecules for blocking suppressive pathways).

New insights into the pathogenesis of HIV-1 infection highlight several new and promising areas of investigation for immune-based therapies, including strategies that target T-cell homeostasis and immune activation, as well as those targeted at restoring immune responses directed against HIV.

The rationale behind the investigation of a variety of cytokines as adjunctive therapies to antiretroviral treatment is to stimulate the immune system and improve HIV-directed immune responses. The potential benefits of cytokine based-therapies in conjunction with HAART include:

1) Stimulation of specific immune responses to HIV-1, such as restoration of T-cell proliferation and IL-2 production;
2) Improved control of viral replication;
3) Increased purging of HIV-1 from viral reservoirs.
Immune based therapeutic approaches current and future directions

Beyond c-ART era, improvement of immune restoration and eradication of persistent HIV-1 cellular reservoirs remain major therapeutic challenges. These goals will require a therapeutic assault on the virus and viral physiopathogenesis from multiple angles. In the future, c-ART might be initiated early in infection followed by strategies to boost the immune system.

**Immunoregulation during Tuberculosis and BCG Vaccination**

Gilles Marchal, Institut Pasteur, France

Different clinical observations suggest a control of asthma symptoms by mycobacteria. Epidemiological surveys report a decreased frequency of asthma in BCG vaccinated subjects.

**Regulatory T Cells Depress Immune Responses to Protective Antigens in Active Tuberculosis**

Am J Respir Crit Care Med 176 pp 409-416 2007

Results in humans

Expansion and function of Foxp3-expressing T regulatory cells during tuberculosis

J. Exp. Med. 204 pp 2159-2169 2007

Results in mice

These results in two species support the hypothesis for Treg expansions by molecule(s) present in *M. tuberculosis*

Clinical trials have shown a significant decrease of asthmatic patients receiving BCG vaccination compared to unvaccinated asthmatic control patients. Recent results have associated increased concentration of regulatory T-cells in blood of patients with tuberculosis compared with healthy control subjects. These results could explain clinical observations of decreased airway hyper-reactivity to acetylcholine observed in patients with tuberculosis.
BCG vaccination decreases asthma

A field observation (Shirakawa, Science 1997) reported a lower frequency of atopic disorders in BCG vaccinated children. This observation was followed by numerous experimental studies in which BCG protected mice against the early and late effects of a subsequent allergenic challenge when given before sensitization to a standard allergen, usually ovalbumin (Ova). (Erb, J.Exp.Med. 1998, or Nahori, Vaccine 2001)

In order to inject larger amounts of BCG than present in a vaccine preparation, without the side effects due to bacterial multiplication and to sensitization against mycobacterial antigens, we killed bacteria by extended freeze-drying (EFD). In order to obtain non viable, killed, BCG without the grossly chemical alterations observed after heat-killing, BCG was extensively freeze dried (EFD) to remove the « totality » of water (< 1.0%).

EFD contains the molecules present in live BCG; it offers the possibility to inject very large amounts of bacterial corpses without the side effects due to bacterial multiplication. The toxic compounds created during heating or irradiation (Maillard derivatives) are absent in the EFD preparation. Since the molecules are not altered by the process, a biochemical characterization of active molecules is possible.

The potency of this preparation to control asthma symptoms was studied in mice and guinea pigs in validated asthma models.

Extended freeze-dried BCG decreased features of asthma when applied once the asthma model is present including established asthma. Extended freeze-dried BCG exerts an immunoregulatory effect on Th2 and Th17 cells through a mechanism relying on expansion of pDCs and Tregs and does not modify ongoing Th1-mediated effector mechanisms. Thus, extended freeze-dried BCG may be effective as an immunoregulatory preparation against asthma without impairing capacity to control infections. EFD controls inflammatory processes in the lung.

On other markers of inflammation, extended freeze dried BCG reduce lung inflammation (cells & cytokines), blocked NF-kB pathway, p38 phosphorylation, reduced COX-2 expression through IL-10 production, and enhanced PPARy.
The specific preparation by freeze drying was more efficacious than BCG itself or heat killed BCG.

EFD does not interfere with *M. tuberculosis* infection: In an assay on guinea-pigs, a species more susceptible to *M. tuberculosis* than mice, EFD was given before, after, or without BCG, respectively. The EFD treatment did not modify sensitization to PPD and did not sensitize to *M. tuberculosis* infection or impair BCG vaccination.

The therapeutic window of EFD being large, its use to treat inflammatory reactions is open.

In conclusion, extended freeze-dried BCG reverses the type 2 cytokine profile characterizing atopic disease and may be effective as an immunoregulatory preparation against asthma and other allergic or auto-immune diseases without impairing capacity to control infections. Moreover, extended freeze-dried BCG by enhancing PPARγ expression and blocking the activation of NF-κB and p38 phosphorylation could be a new therapeutic agent in different inflammatory diseases.

---

**IL-7 a Multifunctional Cytokine**

Michel Morre, Cytheris, France

Interleukin-7 (IL-7) is a multifunctional cytokine/growth factor, mainly produced by non hematopoietic cells, which is active on T cell development, expansion, response and protection from apoptosis. IL-7 has a critical and, at some steps, a non-redundant stimulating effect on T lymphocyte development, notably on thymopoiesis and, downstream from the thymus, on homeostasis expansion of peripheral T-cells.

IL-7 Boosts Immune Response by Acting At Many Levels of Lymphocyte Maturation

Two critical properties justify its development as a drug, both in oncology and infectious diseases:
- A quantitative effect consisting of a massive expansion of T-cells which provides the basis for immune recovery and breaking through tolerance to chronic stimulation by virus or tumor-derived antigens.
- A functional enhancement of αβ-T-cell reactivity to weak immunogens, such as proteins of virus causing chronic infections or tumor-associated antigens.

In addition to the anti-apoptotic effect of IL-7, supported by numerous studies, IL-7 significantly extends the availability of T-cells for specific immune responses.

Two other significant effects of IL-7 have been recently documented or reconfirmed:
- IL-7 antagonizes both the production and the downstream post receptor signaling of TGF-β.
- Through induction of chemokine and chemokine receptor production, IL-7 administration triggers the homing of T-cells to deep lymphoid organs such as spleen, lymph nodes and gut mucosa.

The broad activity spectrum of IL-7 provides an opportunity to develop the product along several lines:
- The restoration of the immune system and the prevention of opportunistic infections in patients who develop severe lymphopenia, such as in HIV infection or treatment with lympho-ablative chemo- or radio-therapies
- The control of chronic viral infections such as HIV, HCV, and HBV or in the treatment of cancers, either in the minimal residual disease or in more advanced tumors, where IL-7 could be used as stand-alone agent or in combination with other immunotherapeutic agents, notably with vaccines or as an adjunct to antiviral drugs.
- In support of vaccines, using IL-7 to boost the response of a vaccine for therapeutic purposes (such as boosting a cancer vaccine) or using IL-7 in older patients or immuno-deficient patients to improve the response to the more classic prophylactic vaccines (such as preparing HIV infected patients for HBV vaccination or preparing older people for influenza vaccination).

Considerable evidence from basic immunology, preclinical models and more recently, from clinical studies, confirms the unique role of IL-7 in the functioning of the immune system and especially in providing the right cells in sufficient numbers to support and improve specific immune responses against infectious agents and malignant cells.
Session 4: Vaccination and Combination Approaches

Blockade of IL-10 Receptor to Treat Persistent Viral Infections
Matthias Von Herrath, La Jolla Institute for Allergy and Immunology, USA

First, some considerations:
The induction of Teff and Tregs occurs during infection, inflammation, autoimmunity
- Regulation is context and likely organ dependent
- Tregs will likely have as many functions and phenotypes as Teff
- The definition of main switch-points will be crucial (i.e. IL-10, TGFβ, FoxP3 and interaction with APCs).

Taking into account the kinetics of the immune response to the LCMV (lymphocytic choriomeningitis virus) model as shown below; studies were performed ex vivo and in vivo to determine the impact of IL-10 production in the chronic viral infection.
LCMV Arm

**Acute infection**
- Antigen CD8 T cell
- Viral clearance
- Memory cells

LCMV Cl13

**Chronic Persistent infection**
- Lack of host immune response
- Immuno suppression
- Viral titers correlate inversely with CTLp

It was observed that during the chronic infection of the LCMV there is a significant increased IL-10 production *ex vivo* (clone 13) but not acute LCMV (Arm) infection.
Studies performed in LCMV infected mice have also shown increase in IL-10 secretion. CD11c/CD8-neg DCs from Cl13 infected mice induce IL-10 secretion by CD4+ anti-viral responder cells.

The following graph shows at a glance how CD8aneg DCs and IL-10+ 'TR1'CD4+ could maintain chronic infections; the immune system potential interactions that take place for chronic infections to be persistent.
Thus, using the (LCMV) lymphocytic choriomeningitis virus murine model of chronic infection, it was shown that treatment with anti IL-10R mAB restores antiviral CD8 responses and drastically lowers systemic IL-10 production, and accelerates clearance of the persistent infection without noticeable side effects.

Anti-IL-10R antibody treatment leads to the resolution of protracted infection, enhances antiviral T-cell responses and abrogates the ability of CD8neg dendritic cells (DCs) to induce IL-10 in CD4 T cells.

These findings are in agreement with decrease of viral titers in mice deficient in IL-10 and underline the importance of IL-10 in maintaining a chronic viral infection.

In detail, IL-10R abolishes CD8neg DC-mediated IL-10 production in responder T-cells early after infection and decreases absolute CD8neg DC numbers over time, thereby inducing a shift towards IFN-y and Tc1/Th1 T cell responses, enabling viral clearance.

In addition, IL-10R decreases PD1L expression on T-cells, thus disabling the negative feedback loop through PD1/PD1L interactions that controls T-cells during chronic infections. Our observations also show that anti-IL-10R antibodies synergize with other immunotherapeutic approaches such as OX40 agonists that can enhance anti-viral T-cell responses. Thus, based on the fact that IL-10 production is increased in chronic HVC, CMV and HIV infections, one can envision IL-10R blockade being part of future combination therapies especially in individuals who do not respond well to established anti-viral strategies.
**Vaccination against CMV Infections**  
Mark R. Schleiss, University of Minnesota, USA

Each of the 8 members of the human Herpes virus family identified to date have been associated, to varying degrees, with congenital and prenatal transmission to the fetus and newborn infant.

Some highlights about the Congenital CMV Infection

- Most common congenital viral infection in the developed world
- Incidence of congenital infection: 0.5-2% of all pregnancies
- A major cause of mental retardation, developmental disabilities, hearing loss
- There is a compelling need for a vaccine (Stratton et al., 1999)

The problem of prevention of mother-to-child transmission of Herpes virus infections is complicated by a lack of understanding of correlates of protective immunity for both the mother and the fetus. Herpes viruses encode multiple immune evasion genes, and establish lifelong, persistent infection in the host. These issues render the design of protective vaccines highly problematic.

In spite of these challenges, progress has been made in recent years toward the development of new vaccines for Herpes viruses, in particular these viruses that pose the greatest risk to the newborn: herpes simplex virus (HSV) and cytomegalovirus (CMV).

Recent strategies undergoing evaluation in clinical trials include both the approach of attenuated, live-virus vaccines as well as recombinant, subunit vaccines that target immunodominant virally-encoded proteins.

In general, the strategies for the CMV vaccines include:

- Envelope glycoproteins: humoral targets
- Tegument proteins and regulatory proteins: CMI and CTL targets
- Expression techniques: adjuvanted recombinant expression systems, vectored approaches
- Live, attenuated vaccines

A recombinant subunit vaccine against genital HSV infection, based on the viral glycoprotein gD, has demonstrated efficacy in placebo-control studies, and deserves further investigation.

Animal models are necessary to study CMV vaccines, the guinea pig CMV is the only rodent model of transplacental transfer to a foetus. This model was used in a study where it was observed that the Cytomegalovirus is transmitted in utero leading to disease in the newborn.

There is evidence that preconception vaccination protects the fetus in the guinea pig model such as: Live, attenuated vaccines (Bia et al., 1980), Adjuvanted native glycoprotein vaccines (Harrison et al., 1995; Bourne et al., 2001), Passive antibody (Bratcher et al., 1995; Chatterjee et al., 2001).

A benefit of immunization against genital HSV for prevention of neonatal HSV infection is inferred, although this endpoint may ultimately be difficult to demonstrate in clinical trials. Progress has also been made for immunization against congenital infection with CMV. Given the neurodevelopmental injury associated with congenital CMV infection, such a vaccine is a major public health priority.

Recently, subunit vaccination with the CMV glycoprotein, gB, has demonstrated efficacy against acquisition of infection in a phase II trial in young women. In a placebo-controlled study, vaccination had a significant impact on the probability of a study participant remaining...
CMV seronegative through the 42-month follow-up-period. These data are the first that demonstrate significant efficacy of a CMV vaccine for prevention of infection, and will drive interest in future gB vaccine studies using other expression technologies. Although these results are encouraging, ultimately, several major issues must be resolved before CMV vaccines can be optimized.

First, the phenomena of re-infection of CMV-immune hosts with new strains must be understood, since non-primary CMV infections account for the major disease burden of congenital CMV. Presumably, vaccine design will need to account for the heterogeneity in clinical isolates that may account for most re-infections. Secondly, the role of viral immune evasion genes in abrogating host immunity must be clarified: a better understanding of this process may, in turn, allow the design of optimized live, attenuated vaccines. Finally, the role of immune responses that may potentially control at mucosal surfaces needs to be elucidated, and this knowledge may in turn facilitate the development of novel vaccines that block primary infection at mucosal sites. Data from a relevant animal model of congenital CMV infection, the guinea-pig model, can help to prioritize which vaccines are likely to be of greatest benefit in clinical trials.