the giovanni ARMENISE HARVARD foundation

ARMENISE-HARVARD SYMPOSIUM 2012

IMMUNOBIOLOGY OF MICROBIAL HOST INTERACTIONS

14th Biennial Symposium
June 10-13, 2012, Borgo San Luigi (Siena), Italy

About the Symposium

Maybe it was the setting which made even usual things look unusual. The beautiful Tuscan countryside and the hills around Siena, covered with grapevines and adorned by the towers of Monteriggioni, were home to the 14th symposium of the Armenise-Harvard Foundation, dedicated to the ""Immunobiology of Microbial Host Interactions"". Between June 10 and 13, the meeting brought together many people who belong to the Boston area biomedical community, and who regularly meet each other at the Harvard Medical School or other scientific institutes in the city. But they still managed to discover new things and learn a lot from each other in Siena, where they presented their newest data. And so did researchers from Italy, Switzerland, even Singapore.

In order to understand the interaction between virus, bacteria and the immune system, you need to work at the intersection of different disciplines: immunology, cell biology, genomics, pharmacology, etc. The talks in Siena reflected this variety of approaches.

All in all, they covered two main territories. On one side, there were people who are working directly on developing new vaccines. They may use immunology and microbiology to prevent the establishment of a disease. This group included Rino Rappuoli, with his fascinating keynote speech which led listeners through different historical stages of vaccine development, from Louis Pasteur to genomics, reverse vaccinology and beyond. Then there were Davide Serruto, Dan Barouch and David Knipe presenting case studies on meningitis, HIV and herpes respectively.

The second group includes researchers who are studying what precedes the development of disease: how different pathogens manage to escape the immune reaction, and sometimes even to hijack it at their own advantage. This kind of research provides information that vaccine developers can then use to counteract the pathogen's strategy.

Some talks focused on basic immunology, analyzing newly discovered cell types that contribute alternatively to host protection or to disease: Lorenzo and Alessandro Moretta discussed natural killer cells. Maria Grazia Roncarolo gave a great review of regulatory T cells. Michaela Gack

described how the influenza virus modulates a particular signaling pathway, RIG-I. Giulio Superti Furga focused on pathways of type 1-interferon induction and how pathogens can manipulate them. Nir Hacohen gave a great view of how Rna interference can be used to test various hypothesis about the immune system. Further on, John Mekalanos showed a detailed structural and functional analysis of a chemical weapon some pathogens use to ruthlessly kill their competitors, and Eliana Coccia described the factors responsible for tuberculosis virulence, which might soon help the development of an effective vaccine. On the opposite side there were studies focusing on the immune effectors, the molecules which make up the artillery used by the immune system to spot and fight pathogens. Anna Rubartelli updated her colleagues on the role of IL1beta. Carla Castagnoli showed IL 2 produced by dendritic cells is important not only in adaptive immune response but also has a less known role in stimulating the innate response. Dennis Kasper reminded everyone of the importance of the bacterial flora, and of the fact that every species should stick to the flora it is born with. To make the point clearer, Wendy Garrett showed what happens when the flora is disrupted, opening the way to pathogens and possibly even tumor development. Amazing real time, real tissue views of how the immune cells responds to pathogens in vivo were shown by Matteo Iannacone (using VSV) and Thorstein Mempel (HIV). Federica Sallusto made sure no one forgot about adaptive immunity with a big science approach. Judy Lieberman wrapped up the meeting by showing cool, hitherto unknown ways in which HIV avoids innate immunity.

Humans and their pathogens are constantly at war at each other, and evolution constantly provides microbes with new tricks with which they can bypass our immune system. But humans have science on their side, and as the Siena meeting showed, it is a powerful line of defense.

Vaccines, Medicine and Public Health in the XXI Century

Rino Rappuoli

Novartis Vaccines and Diagnostics, Siena, Italy

The task of opening the 10th workshop of the Armenise Harvard Foundation fell to Rino Rappuoli, one of the world's leading experts on vaccines. Born near Siena and Global Head of Vaccines Research for the Siena-based Novartis Vaccines & Diagnostics, Rappuoli was the ideal choice to play host to a meeting, which gathered more than 20 top researchers in the Tuscan countryside to explore the future of immunology and vaccine development.

For a start, Rappuoli drew inspiration from one of the most notorious views of his hometown, the cathedral of Siena. Its construction began in the 13th century, with plans to build the largest cross-shaped cathedral in the world. But in 1348 the plague arrived in the Tuscan city, which was hit hard and never recovered. Works on the cathedral had to be interrupted, and were later resumed in a less ambitious form: the Dome was eventually built smaller, and what was originally supposed to be the short arm of the cross became the main aisle. A wall at the back of the Cathedral is all that is left of the original plan to extend the church, and it stands as a monument to the devastating power of infectious diseases in human history. But that was not the only time Siena had to face a pandemic. Like many other cities in Europe, it was hit hard by smallpox in the 18th century. At the time this terrible infectious disease, now eradicated, killed more than 600 000 people a year in Europe (whose population at the time were only 80 million people). Siena is home to an antique and glorious scientific academy, Accademia dei Fisiocritici, which since 1760 publishes a scientific journal. Its first volume was recently reprinted in the occasion of the Accademia's 250th anniversary, and is entirely dedicated to smallpox. Rappuoli noted how it described variolation, the first attempt at some form of vaccination towards infectious agent. It consisted in deliberately infecting a person with a small quantity of substance taken from the pustules of an infected person. The journal examined twenty cases, describing in full details what followed: nothing for a few days, but after one week things become serious. The patient had pain, fever, shivering, which grew more and more violent and went on for about 30 days before the blisters disappeared. ""When we ask why some people are scared of vaccines now"" Rappuoli notes, ""we should remember that they started as a very unpleasant thing"". Still, they are much better than the disease.

The real scientific development of vaccines started of course with Louis Pasteur, who at the end of 19th century established the three core scientific principles of vaccine development: in order to vaccinate against a disease, you need to isolate, inactivate and inject the pathogen, be it a bacterium or a virus. And that's what immunologists have been doing for more than a century. This strategy has allowed produce very successful vaccines, which wiped smallpox out of the planet and eliminated polio from most of it. Vaccination, when it works, reduces a disease's mortality by 97 per cent. Even the best therapy can do no better than 75 per cent. So, vaccines are by far the most effective medical intervention ever developed.

Yet Pasteur-style vaccines now look like a very nice but very old technology. Why should we still be excited about vaccines, then? Actually, many people are, including top scientific journals, which have been running cover stories about vaccines in the last few years, and big companies, which are investing big sums of money on their development. And the reason is that, starting with the 1970s, subsequent waves of new technologies have made entirely new things possible, opening up a totally new era for vaccines.

A few case histories told by Rappuoli go to show how new technologies have allowed vaccines to go beyond the ""Pasteur"" era.

The first one is about meningococcus. This bacterium has an outer capsule made of repeating units of sugars, and already in the 1960s it was shown that if you have antibodies for this capsule you are protected from the infection. Vaccines were made from purified sugar capsules, but they worked well only for military purposes. They did not work very well in infants, and the immunological memory was too short, making them at best useful for controlling sudden outbreaks.

Then conjugation technology came along. By linking sugars to a carrier protein, you finally get a vaccine that works. In 2000 a conjugate vaccine for meningococcus C was licensed and used to vaccinate the entire population under 18 years of age in Great Britain. The disease disappeared in a year and has been gone ever since. Conjugate vaccines are also a good example of how new technologies create new theories, which in turn can help refine those same technologies. For 20 years scientists believed that the lack of an immune reaction to the sugars in the capsule alone was due to the fact that T cells cannot recognize them, and can only be activated by peptides in the protein carrier. Until, last year, Dennis Kasper (one of the workshop participants) proved that there is actually a population of T cells which are specific for the polysaccharide portion of the vaccine and that the peptide is only needed as an anchor-like sugar: a notion that can now be used to potentially enhance the efficacy of the vaccine.

The conjugate technique has allowed the development of vaccines for all strains of meningococcus except the "B" one. The conjugate vaccine does not work for this one, because repeating unit happens to be a polysialic acid which is too similar to a human polysialic acid to induce an immune response. To solve this problem, a new technology was needed, namely genomics. The whole genome of the meningococcus B was sequenced, and scanned in search of new antigens, which could be used to make a vaccine. Rappuoli's group managed to obtain a vaccine based on three antigens, which has been through phase I, II and III studies in different age groups, and is now awaiting license. So, the combination of conjugate technology and reverse vaccinology (genomics) has provided us with all the tools to eliminate all known pathogenic meningococci. The same techniques are being applied to antibiotic resistant bacteria such as staphylococcus, (phase I), or to Escherichia coli, a particularly tricky pathogen because it comes in six different strains. Rappuoli's group is scanning their genome in search of common antigens that would allow a vaccine that is effective against all known variants of E.coli.

The next wave is structural vaccinology: the study of molecular structures to manipulate antigens and make vaccines that would otherwise not be possible. One good example is the attempt to stabilize the fusion protein of respiratory syncytial virus to make a vaccine. Previous attempts to a vaccine against this virus, which normally affects children, did not work because the protein used as an antigen changes all the time. But with structure -based vaccine it is possible to make a stable mutant that can be used for a vaccine. Once again, the same approach can apply to other virus, in particular HIV or influenza strains.

Finally, adjuvants (compounds added to the vaccine to increase its efficacy), which used to be the black box of immunology (they have been used for decades, and they work, but nobody really knew why) are booming real science. Their importance became clear during the 2009 H1N1 outbreak. Only the study and use of adjuvants made possible for Novartis to produce the required doses of vaccine in such a short time: a good adjuvant not only allows a lower quantity of vaccine per dose, but also improves the response.

All these new technologies not only make vaccines more effective, but also more specific and more defined. They also allow us to think about vaccines in a totally new way. Our society, after all, has changed a lot since the golden age of Pasteur-style vaccines. Vaccines were originally developed for a society with many children and few elderly. Now, in most western countries at least, fertility rates have dropped, whereas life expectancy has increased and continues to do so. Every 10 years, it grows by 2,5 years (of course a lot of this increase is itself the consequence of having less infectious diseases). So, vaccines now have to address the needs of new age groups. Maternal immunization can become a key issue. It is clear from epidemiology that vaccinating pregnant women (against tetanus and influenza, for example) can drastically reduce complications and mortality among newborns. Adolescence has it specific needs (think of sexually-transmitted diseases). But the age group that can benefit the most from new generation vaccines are the elderly. They could use more effective vaccines against Influenza, herpes zoster, hospital infection such as pneumococcus and antibiotic resistant bacteria. And, of course, cancer-preventing vaccines, a relatively new but extremely promising field. Developing countries, on the other hand, still need a lot of "classic" vaccines, as well as new ones for diseases which have not been dealt with yet.

There, Rappuoli believes, vaccines can actually help fight poverty. In the developing world, infectious diseases are not only caused cause by poverty, but actually contribute to it. For example, a family where someone has meningitis ends up spending most of its outcome in caring for the diseased. The problem, of course, is that researchers do not easily get money to make vaccines that have no big market. They can have funding for doing basic research, but developing a vaccine and going through clinical studies are another matter. That is why Rappuoli is setting up a non-profit institute in Siena, with the same technologies available to major pharmaceutical companies but entirely devoted to developing vaccines against diseases in poor countries.

Exotoxin A Triggers an Immune Response in *Caenorhabditis elegans*

Frederick M. Ausubel

Department of Genetics, Harvard Medical School, and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114 USA

Friend or foe? Every time the cells of our guts meet a microbe, they have to ask themselves this question. The human digestive trait regularly hosts dozens of species of bacteria. A lot of them are not only harmless, but actually essential to our survival, and researchers call them our "commensals". But other microbes can be extremely dangerous and cause infectious diseases. How can the immune system recognize what kind of microbe it is facing, starting a response in one case and letting the commensals thrive peacefully in our guts on the other? Fred Ausubel's current research at Massachusetts General Hospital revolves around this question. The most obvious hypothesis would be that our immune system can recognize the microbe's "dress", the specific molecular patterns found on the microbe's outer surface (they are called "microbe-associated molecular patterns" or MAMPs). The problem is that in many cases both friendly and unfriendly bacteria wear the same clothes: they have the same MAMPs, so there must be something else that tells the immune system when to act. A suggestion might come from plants, which are known to recognize pathogens not so much from their own features, but from the effects they cause on the organism: for example, the specific way a pathogen alters the chain of chemical signals in a plant may function as an alarm signal.

Ausubel and colleagues wanted to check if something similar might be working in animals as well. Their model organism was the laboratory worm par excellence, C. elegans. This animal has a very simple immune system, whose action is mediated by intestinal epithelial cells (IECs). The worm can be attacked by a number of pathogens, and the best studies are the human opportunistic pathogen Pseudomonas aeruginosa, which causes a strong immune response. On the contrary the common laboratory bacterium Escherichia coli are harmless to the C. elegans worms. To test the idea that the immune reaction is triggered not by the bacterium itself, but rather by something it does, the researchers engineered a version of E. coli which produces the potent toxin Exotoxin A (ToxA), a protein Pseudomonas aeruginosa uses to block the production of proteins in the worm, thus killing it.

What they found was that ToxA synthesized in E. coli induces a strong immune response by activating a group of genes that are normally induced when the worm is infected with P. aeruginosa. But that only leaves us with another question: how is ToxA recognized? This could happen either directly, or via the damage the toxin causes to the organism. It turns out the latter is true. The researchers were able to prove it by engineering a version of ToxA that is enzymatically inactive; i.e., the toxin had the same molecular ""dress"" but was unable to start a chemical reaction that blocked protein synthesis. In this case, the ToxA protein failed to activate an immune response. And the same thing happened when the researchers engineered a C. elegans that, due to some mutated genes, was unable to synthesize the diphthamide residue, a modified amino acid which is the main target of ToxA. Again, the worm's immune system did not respond to the toxin. On the contrary hygromycin, a protein which blocks the translation of RNA into proteins, activated an immune response that was similar to the one activated by ToxA. Ausubel's conclusion is that, similarly as it happens in plants, what really triggers the C. elegans immune response to ToxA is not the toxin itself but its main effect: namely, the blockage of protein production.

The Inflammasomes and IL-1b Secretion in Health and Diseases

Anna Rubartelli

IRCCS

Autoinflammatory diseases are a relatively new field of study for immunology. They are chronic diseases which specifically affect the innate immune system - unlike better-known autoimmune diseases such as lupus or rheumatoid arthritis which are mainly due to malfunctioning of the adaptive system - and they are characterized by periodic, intense episodes of inflammation that result in such symptoms as fever, rash, or joint swelling.

Researchers have long suspected that interleukin-1beta (IL1b) plays a role in this class of diseases. IL1b is cytokine (a protein used by cells to communicate with each other) with unusual features. When IL1b was discovered in 1984 it was not clear how it is produced in its active form and secreted. The discovery of the inflammasome (an aggregation of different proteins found in myeloid cells which are part of the innate immune system) shed light on this dilemma, demonstrating that generation of active IL1b requires cleavage by the activated inflammasome. But what activates inflammasome? We do not know much about it, and Anna Rubartelli at the Cell Biology Unit of the IRCCS AOU San Martino - IST in Genoa, is trying to settle the matter. In spite of the clear definition of its molecular components, how and where the inflammasome gets activated is still largely unknown. A role for the reactive oxygen species (ROS) in activation has been proposed but subsequently questioned - evidence exists both for and against a role of ROS in inflammasome activation.

On the other hand, the regulation of oxidation in the cell always works as a balance: whenever ROS build up because of stress factors, they are immediately contrasted by anti-oxidant factors. if the balance is lost the cell undergoes oxidative stress. So in a cell it can be difficult to discriminate whether ROS or antioxidants are responsible for a given phenomenon. The researchers came up with the idea that both (or, better, the right balance of the two) are actually required for inflammasome activation. In fact, if you block either ROS production or antioxidant production you get the same result, namely no more IL1b.

In order to check whether inflammasome and IL1b are involved in autoinflammatory diseases, Rubartelli and colleagues worked on cryopyrin associated periodic fever syndromes (CAPS), diseases characterized by severe inflammatory symptoms such as fever, rash, arthritis, growth delay, neurosensorial defects. They found that the overall increase in IL1b secretion in CAPS cells compared with healthy controls is modest, but the secretion is much faster. The maximum level is reached in 3 hours and (not in 24, like in physiologic conditions), as if all IL1b is secreted at once.

The researchers then checked the balance of oxidants and antioxidants in CAPS monocytes freshly drawn from the blood, and found they have a higher content both of ROS and antioxidants compared to healthy cells. They are still in equilibrium but much more precarious than healthy cells. The problem is that when these cells are triggered by inflammatory stimuli, antioxidants collapse and expose the cell to oxidative stress. When this happens, CAPS monocytes do not die, but

show clear signs of stress, such as damages to the mitochondria (small organelles that are the cell's power plant).

At this point, the question for the researchers was: does this stress in CAPS monocytes influence the production of cytokines downstream of IL1b, first of all IL-1 Receptor antagonist (IL-1Ra) and IL6? The question is not trivial, since IL-1Ra is the natural inhibitor of IL-1 activity, and is required to down modulate inflammation also in healthy individuals. Lack of IL-1Ra allows unopposed action of IL-1 with dramatic consequences. In turn, IL-6 is a ""bridge cytokine"" between innate and adaptive immunity.

The researchers demonstrated that the stress state of activated CAPS monocytes is indeed responsible for a reduced secretion of cytokines downstream of IL-1. The deficient secretion of these cytokines coupled with increased IL-1β release explains the severity of the IL-1-related clinical manifestations and the predominant implication of innate immunity in CAPS.

Species-Specific Inhibition of RIG-I-Mediated Interferon Induction by Influenza A Virus

Michaela U. Gack

New England Primate Research Center, Harvard Medical School, Southborough, MA

It is one of modern medicine's worst nightmare scenarios: a genetic mutation suddenly makes the influenza A virus capable of jumping to humans from another species, finding our immune system unprepared and causing a global pandemic similar to the one that swept the world in 1918. Luckily, in the recent outbreaks caused by the H5N1 strain (better known as avian flu) the virus did not learn how to pass from human to human efficiently. In contrast, the H1N1 virus (known as the swine flu) could easily transmit from human to human but in the majority of cases did not cause death.

Understanding how this family of viruses can adapt to new species is crucial if we want to learn how to defend ourselves and prevent a catastrophic pandemic. At the Primate Research Center of Harvard Medical School, Michaela Gack is focusing on a key mechanism that influenza viruses use to silence the host's immune system. In mammals, the innate immune system includes a family of sensors for virus detection, called cytosolic RIG-I-like receptors (RLR). One of the proteins of influenza virus called non-structural protein 1 (NS1) can block the function of one of these sensors, RIG-I, from working properly. More precisely, the viral NS1 protein blocks the ubiquitination of RIG-I, which is necessary for producing antiviral factors, called interferons, and thus to alert the immune system of the virus' presence. Ubiquitination is the process by which certain proteins are tagged with a molecule called ubiquitin and thus marked for destruction; however in the case of the sensor RIG-I, ubiquitination does not lead to its degradation but is needed for its function to alert the immune system after virus infection. By blocking the ubiquitination of RIG-I, the viral NS1 protein does what thieves do upon entering a house: they turn off the alarm system. The viral NS1 protein manages to do so by binding to the protein TRIM25, which mediates the ubiquitination of RIG-I. This is confirmed by experiments using mutant versions of NS1 that lose the ability to bind to TRIM25. Recombinant viruses carrying these NS1 mutants had a much lower ability to replicate in lung cells due to their inability to block RIG-I.

Gack and colleagues have thus studied how different NS1 proteins from avian, human, swine and mouse-adapted influenza viruses can interact with mammalian and avian TRIM25 proteins. These studies are important to assess what factors can facilitate or block the transmission of the virus from one species to another. What they found was that the mechanism by which NS1 works is strikingly different in the case of mice. Human TRIM25 binds to all tested NS1 proteins, whereas the chicken TRIM25 binds preferentially to the NS1 from the avian virus. But none of the NS1 proteins were able to bind mouse TRIM25. This was puzzling since NS1 can still do its job and inhibit interferon production in mouse cells. Gack and colleagues hypothesized that NS1 blocks interferon production in mouse by another mechanism which does not involve TRIM25. They were able to show that in mice the viral NS1 targets another protein called Riplet, which is also able to ubiquitinate RIG-I. Interestingly, some of the human influenza viruses have evolved to block both TRIM25 and Riplet in human cells for the potent suppression of the host’s immune system in humans. Influenza viruses can thus develop different mechanisms in different species to fight the innate immune system, which helps to explain the ability of these viruses to adapt to a number of different host species.

Human Natural Killer Cells: From the Biology to Clinical Applications

Lorenzo Moretta

Istituto Giannina Gaslini, Genova

Natural Killer cells are the patrol police of innate immunity. They play an important role in tumor surveillance and in defenses against viral infections by wandering around, looking for cells that appear abnormal or infected, and ruthlessly killing them. Lorenzo Moretta and colleagues have found and tested a why not only to make them more efficient, but also to use them to cure high-risk leukemias (leukemia is a type of cancer that affects blood cells).

On their outer surface, NK cells have inhibitory receptors that allow them to recognize Human leukocyte antigens (HLA) that are proteins normally found on healthy cells: one of their function is to allow NK cells to recognize healthy cells and leave them alone. NK cells have inhibitory receptors for various HLA alleles, discovered by Alessandro Moretta over 20 years ago. These receptors are tuned to recognize different groups of HLA variants alleles which are characteristic of the individual. HLA molecules are part of the Major Histocompatibility Complex, a group of genes and related proteins which allow the immune system and, in particular T cells, to recognize foreign antigens. Notably, most tumor cells lose one or more HLA class 1 molecules and become susceptible to NK-mediated attack.

At the same time, NK cells also have activating receptors (another important discovery of the Moretta's group) which in turn do recognize surface molecules expressed by stressed cells, tumor cells in particular. Not all normal cells manage to escape the attack of NK cells though. In particular, dendritic cells that failed to undergo proper maturation have such low levels of HLA molecules on their surface that they can be attacked by NK cells.

Lorenzo Moretta's work is focused on exploiting this mechanism to improve the efficacy of bone marrow transplantation (or, more precisely, hematopoietic stem cells transplantation) to cure aggressive leukemia. In particular, his group is focusing on haploidentical hemopoietic stem cells transplantation, the situation where only one of the HLA-carrying chromosomes is matched with that of the patient while the other is mismatched (this typically happens with parents and about 50 per cent of the siblings). The key that makes this kind of transplants more effective against leukemia, preventing both tumor relapses and immune reactions which compromise the outcome, are alloreactive NK cells, i.e. NK cells from the donor which, being tuned to different HLA alleles, do not spare the patients' leukemia cells. Alloreactive NK cells derive from transplanted hemopoietic stem cells, mature in the patient's bone marrow and can target and kill leukemia cells and prevent relapses. But they can also prevent graft-versus-host reaction (the case when cells from the donor's immune system, in particular T lymphocytes, start to attack the patient's organism). This latter function is due to NK cells killing the patients' dendritic cells, which are known to initiate T cell - mediated GVHD presenting antigens from the host to T cells from the donor, And if it not enough, by killing the patient's T cells alloreactive NK cells can also prevent the rejection of the transplant by the host's immune system. A novel and interesting experimental approach was tested in a mouse model with promising result. Indeed, it was shown that the infusion of mature, alloreactive NK cells in mice prevented GVHD to such an extent that mice that were given these cells could receive mismatched bone marrow grafts containing up to 30 times the dose of cells which is usually lethal, in the absence of any evidence of GVHD. Transferring this approach to humans is challenging but possible, and Moretta's group at Ospedale Gaslini in Genova is working to turn the idea into a clinical protocol.

An Innate, Antibody-Independent, Antiviral Role for B Cells

Matteo Iannacone

San Raffaele Institute

If you ask an immunologist to think about the main ingredient making our immune system work, a probable answer would be "the B cells". It's a group of white blood cells playing a crucial role in the adaptive immune system, the one giving our body the ability to recognize and remember specific pathogens each time they are encountered.

This first-line response mechanism is the one allowing the immune system to fight off infections. And B-cells are thought to produce the antibodies needed for this vital function. But is this a universal rule? Yes and no, says Matteo Iannacone of the Harvard Medical School. His strongly innovative research approach revealed some properties of B cells that were previously unknown. In particular, he showed that if they are essential for survival in some infections, the same cannot be said of the antibodies they produce.

With his research team, Iannacone studied the response of mice to neurotropic vesicular stomatitis virus (VSV), a member of the same family as rabies. VSV is common in livestock and rodents, and can cause flu-like symptoms in humans.

Experiments showed that mice infected with VSV could suffer fatal invasion of the central nervous system even as they generated a high concentration of anti-VSV antibodies in their system. This observation led researchers to revisit the contribution of adaptive immune responses to survival following VSV infection. They studied VSV infection in B cells-deficient mice and in transgenic mice that had B cells but did not produce antibodies.

Unexpectedly, while the former succumbed to VSV infection, the latter were completely protected. So survival after VSV exposure depends on B cells, but does not require antibodies or other aspects of traditional adaptive immunity. In this case, the role of B cell is to protect the host cell from VSV infection by assuring that a particular type of cells, lymph node macrophages, produce cytochines molecules. Cytochines are small cell-signaling interferon proteins, able to prevent the virus from invading the central nervous system. This function does not depend on antibodies at all. These results challenged the idea, called by Iannacone "a gospel", that the neutralizing antibodies (antibodies specialized in the inhibition of infections' biological effects) are absolutely needed for protection against this infection.

The research project is continuing in Iannacone's own Lab, a Giovanni Armenise-Harvard Foundation Laboratory at the San Raffaele Scientific Institute in Milan, Italy. In the future, it may shed new light on the prevention against potentially lethal viruses.

Migration of HIV-1-infected T cells supports local and systemic viral dissemination

Thorsten R. Mempel

Massachusetts General Hospital

Many virus and bacteria have found ways to hijack their host cells, including cells of the immune system, so that these actual start working in the interest of the pathogen. HIV, the virus that causes AIDS, is no exception. The main target cells of HIV are T cells, a group of white blood cells that play a key role in cellular immunity by directing the activities of other immune cells against pathogens. The virus binds to certain molecules on the surface of T cells (namely CD4 and either CCR5 or CXCR4), injects its contents into them and sets off a process that leads to the assembly and release of new virus particles. When HIV enters the organism, typically through the mucous membranes of the genital tract or the rectum, the virus faces the challenge that its target T cells are scattered in the tissues of the entire body and it has been unclear how virus particles manage to travel to come into contact with the T cells they end up infecting. This includes the journey from the mucous membranes to the draining lymph node, and from the draining lymph node on to remote lymph nodes and other tissues of the body. Clues came from the observation that, in the culture dish, HIV can jump from uninfected to infected T cells via so-called 'virological synapses', stabilized sites of local contact between the cellular membranes of infected and uninfected cells. But normally, T cells move quickly and constantly and do not frequently come in contact with each other long enough to form these synapses. Thorsten Mempel suspected that the virus uses infected T cells to travel though the body, but at the same time somehow makes their migration less erratic, so that they would tend to form more stable contacts with each other.

To test this idea and to work out how HIV spreads in the body of an infected person, Mempel and his group at Harvard Medical School used so-called humanized mice (mice equipped with what is essentially a human immune system that are susceptible to infection by human viruses) and a technique called multiphoton intravital microscopy to visualize the behavior of their human T cells after infection with HIV in live animals. The researchers used an HIV strain modified to express a fluorescent protein, so that infected cells were easily recognizable, and recorded short movies showing the movement of infected T cells in lymph nodes. The results were just published in the journal Nature. Mempel and colleagues first observed that infected T cells indeed continued to migrate, which allowed them to uniformly distribute within the lymph node closest to the site of injection. However, they found that HIV-infected T cells migrated at lower speeds than their healthy counterparts. In addition, a good share of the HIV-infected T cells formed long and thin extensions, sometimes with multiple branches. The researchers suspected that the HIV envelope protein, which is expressed on the surface of infected T cells before they release new virus particles, might cause infected cells to form tethering contacts and possibly also fuse with uninfected cells, producing these extensions. This was confirmed by a series of experiments showing that many of the elongated cells contained multiple nuclei, suggesting they had been formed by the fusion of several cells. Finally, the group showed that when HIV-infected T cells were prevented from leaving the lymph node closest to the site of HIV entry, the infection did not or only very inefficiently spread to other tissue of the body.

Thus, the infection of migratory immune cells such as T cells appears to be a strategy that allows the virus to overcome anatomical barriers and to shield itself against the perils of the immune defenses that reside in body fluids outside of the cells they infect, such as antibodies.

Innate Anti-Viral Molecular Networks

Giulio Superti-Furga

CeMM, Vienna

“The recognition of viruses by the cell machinery is mostly an interaction game"". Giulio Superti-Furga, Scientific Director of the Research Center for Molecular Medicine of the Austrian Academy of Sciences, explained that to the Armenise Harvard foundation workshop audience when presenting his last promising study in the field.

With his team, he realized one of the very first comprehensive surveys of the antiviral defense tactics of human cells. The attack strategies of 30 well-known viruses were analyzed and compared, and results may advance the development of new antiviral therapies.

Researches focused on the weak points of the innate immune system, the one calling the first-alarm nonspecific body's defense from almost any acute infection.

For viruses to spread, they must enter host cells and hijack them to replicate, assemble and propagate. Because of the restricted size of their genomes, they had to evolve efficient ways of exploiting host cell processes to promote their own life cycles. Once they insert their own genetic information into the host cells, viruses exploit the host cells' molecular machinery, facing and trying to overturn immune defense mechanisms.

Superti-Furga and colleagues discovered that different viruses follow different strategies during their attack: some use camouflage, disguising and masking them to avoid being recognized; others block communication pathways within the host cell, and prevent the immune defense from raising the alarm.

From the 30 analyzed virus types (including poxviruses, herpes viruses, influenza virus and hepatitis C virus) scientists gathered 70 viral genes known to modulate the immune response. They then used mass spectrometry and bioinformatics to identify all the human proteins targeted by the viral products of the inserted genes. A protein complex is a dense package of information, so it allowed to deeply analyzing the viral-human protein interactions.

And "interaction" was exactly the keyword enlightened by this research: the innate immunity involves complex cellular networks, from the detection of pathogens and danger signals to the distinction between self- and non-self-molecules.

In figurative words, researchers allowed the enemy to invade the host cells in order to pinpoint the weak points in the immune system's defense.

579 host proteins were mapped using the viral proteins, leading to an unexpectedly large number of signaling pathways and cellular processes. This suggested yet unknown mechanisms of antiviral immunity: viruses target a much wider set of cellular processes than was previously anticipated.

In the near future these findings may advance the development of antiviral therapies, in particular the design of new target-specific treatments. Weak points in the innate immune system would therefore been strengthened.

The Role of Regulatory T Cells in Immune Responses: A Double-Edged Sword

Maria Grazia Roncarolo

San Raffaele Institute

The immune system is constantly on the lookout for bad guys – from cellular defects to mutant cells, from viruses to parasitic worms threatening the organism's healthy tissue. This seek-and-destroy mission status is made possible by the so-called effector T cells. However, effector T cells can also cause inflammation and damage, and need to be highly regulated by the so-called regulatory T cells or "Tregs". Regulatory T cells are the good guys, immune cells playing a key role in promoting and maintaining immune tolerance and immunological homeostasis.

But how do these guardians work? Maria Grazia Roncarolo, Scientific Director of the San Raffaele Institute of Milan, Italy, says they act like a double-edged sword.

Her recent studies have proved that T cells play a beneficial role in preventing organ-specific autoimmune diseases (diseases that happen when the immune system starts attacking the cells of a particular organ, mistaking them for strangers) and immune mediated rejection of organ and cell transplants; but there are a few cases where Tregs play a negative role, for example in infections mediated by bacteria, viruses or parasites, or in immune responses against cancer cells.

To explain that, her research group decided to look at Tregs in a very peculiar setting: human genetic disease.

In particular, they studied patients with IPEX Syndrome (Immunodysregulation Polyendocrinopathy Enteropathy X-linked Syndrome); a rare disease linked to the mutation of the protein FOXP3, partially responsible of the immune system responses.

Tregs are involved on the frontline in this pathology, since IPEX Syndrome leads to autoimmunity due to the dysfunction of natural regulatory Tcells. In other words, mutations in the FOXP3 gene in these patients cause the dysfunction of the natural Tregs which normally are generated during T cell differentiation in the thymus: the consequence is that the body's immune system attacks the body's own tissues and organs.

Dealing with autoimmune diseases, Roncarolo and colleagues focused on the study of a specific type of Tregs, the adoptive regulatory T cells called Tr1 cells. Tr1 cells are specialized in the inhibition of unwanted immune responses, and their role is to induce tolerance against certain self-antigens and mostly against “nonpathogenic” antigens.

Results showed that Tr1 cells play a role in suppressing the development of autoimmunity and of chronic inflammatory diseases.

These Tr1 cells with suppressive function can be also generated in vitro, so they may become a powerful weapon against IPEX Syndrome, and immune diseases in general.

But this is a slippery slope: when Tr1 cells are induced too early or with higher frequency, they can lead to immunosuppression with consequent chronic infections and lack of immune surveillance against tumors.

Roncarolo explains that with the double role of Tregs: they can be either good or bad, depending on how and when they are induced, and display their suppressor function, it is very important to dissect the signals which regulate this very delicate balance between their beneficial and their detrimental role.

In addition, the future challenge is to identify novel drugs, which can up or down regulate the development and function of these Tregs. By exploiting them in the right way, the next step could be the prevention and cure of immune mediated diseases such as genetic and acquired autoimmune diseases, and chronic inflammatory diseases but also the boost of immune responses against infectious pathogens and tumors.

Vibrio Cholerae-Host Interactions: Nanomachines to Novel Small Molecule Immunomodulators

John Mekalanos

Harvard Medical School

"A view to a kill". That is how, quoting the title of a James Bond Movie, John Mekalanos described the subject of his recent research at Harvard Medical School, the results of which he presented in Siena at the Armenise workshop. The main scene in his talk was in fact a video showing a deadly fight between two microbes, Pseudomonas aeruginosa (a bacterium which causes inflammation and sepsis in humans) and Vibrio cholerae, the well-known bacterium which causes cholera.

Bacteria live in perpetual warfare with each other, battling for the dominion of their territory, i.e. the cells of the host they infect. Some bacteria use a particular weapon, which Mekalanos and his group first described in some strains of Vibrio cholerae: it is a protein complex called Type 6 Secretion System (T6SS), but Mekalanos describes it more vividly as a "spring loaded poison dagger". The researchers tagged some of the proteins which make up this system with the green fluorescent protein (GFP), a protein derived from a jellyfish which allows researchers to visualize and track proteins in a living organism. This way, they could take a look at the T6SS system in living cells, and saw that it is an elongated structure that first assembles, then rapidly contracts and finally disassembles just as quickly.

In even more detailed views obtained by electron cryomicroscopy, which enables researchers to study individual cells slice by slice and visualize them in three-dimensional renderings, the T6SS appeared as a tubular structure anchored to the membrane, made by an outer sheet and an inner tube. Following some unknown signal, the outer sheet contracts, pushing the inner tube out of the cell. It is the poison dagger that hits neighboring cells.

At this point, the whole system disassembles, only to reform some seconds later in a different part of the cell. The tubular structure is formed by two proteins produced by the T6SS gene complex, called VipA and VipB, whereas a third protein, called ClpV, takes care of dismantling it when the work is done. ClpV has no affinity whatsoever for the tube in the elongated conformation. But as soon as the tube contracts, ClpV jumps on, binds it and quickly disassembles it. Presumably upon contact between two cells, the T6SS can puncture and kill another cell (for example, in Mekalanos' experiments, an E. Coli cell), by injecting lethal toxins.

This machinery was first studied in Vibrio cholera. Another human pathogen, Pseudomonas aeruginosa, has a T6SS system too, but it works a bit differently. As Mekalanos' videos clearly show, when several bacteria of this species are grouped together, they adopt what looks like a ""kissing behavior"". Sister cells regularly come in contact with each other and, upon doing so, they occasional assemble a T6SS apparatus, causing the cell they ""kiss"" to do the same. It really looks as if P. aeruginosa can sense T6SS activity in sister cells, and respond to it by assembling their own T6SS system, by translocating protein components at the site of contact. No such kissing is seen in Vibrio cholera cells.

At this point Mekalanos and his group became curious to know what happens if you put Pseudomonas aeruginosa with other bacterial species. First, with Escherichia coli. It turns out, P. aeruginosa leaves E. coli alone. Second test: Pseudomonas sharing space with Type 6 negative vibrio choleare (a strain which does not express T6SS). The two mingle, and nobody is having any

problem. But if V. cholera is of the Type 6-positive sort, than it gets smoked by Pseudomonas. It is not that V. cholera cannot use its T6SS: when it is left alone with E. coli, it attacks and kills it in a few hours. But Pseudomonas is better and faster than Vibrio cholera at using its spring loaded dagger.

Since the T6SS system is so important for the virulence of several bacteria, Mekalanos hopes that its comprehension may lead to the development of drugs that inhibit T6SS activity.

Functional and Immunological Characterization of Neisseria Meningititis

Davide Serruto

Novartis Vaccines

The story told by Davide Serruto is a typical example of how vaccine technology is now going beyond its tradition to tackle new medical problems. It concerns Neisseria meningitiitis, a bacterium which is a major cause of septicemia and of meningitis in children and adolescents. It hits about 3 people in 100.000. It is an exclusively human pathogen, which colonizes the respiratory tract, enters the blood causing sepsis, and then under some conditions crosses the blood brain barrier and infects the brain causing meningitis. It is a terribly fast disease. Data from emergency rooms show that one third of deaths caused by this pathogen happen within 6 hours from the first symptoms. This is too short a time for most therapies, and the best way to prevent deaths is definitely a vaccination.

Immunology-wise, the complement pathway (a cascade of small proteins, part of the innate immune response, that support antibodies and pathogen-eating cells in protecting the organism) is particularly important for meningitis, and a malfunctioning in this system is often associated with increased susceptibility for this disease.

The bacteria come in different varieties, and whereas vaccines have been developed for the A, C, W and Y serogroups, we have no vaccine for the serogroup B, because the capsule polysaccharide which encloses the bacteria is structurally identical to a human self-antigen. So researchers at Novartis applied genomics to circumvent the problem.

They sequenced the genome of the bacterium, and used bioinformatics tools to predict the localization of putative protein antigens on the surface of meningococcus B. They found 28 new proteins that in mice could produce bactericidal antibodies. Since it would be impossible to produce a vaccine with 28 antigens, they then prioritized the antigens and selected the three that were more conserved (i.e. that recur more often across 85 bacteria strains that represent mot of meningococcus diversity in the world) an able to induce a bactericidal activity across diverse strains. They found that 78 % of the strains are killed by a serum raised by the combination of three selected antigens. A vaccine based on those protein antigens is now in the last stages of development.

Yet having a vaccine that works is not enough for 21st century immunologists, who unlike their predecessors also want to know exactly why and how it works. What is the function of those three antigens? The first one is the factor H binding protein (fHbp), a lipoprotein exposed on the surface of the bacteria, which can bind to the human Factor H. The researches created a knockout version of the bacteria deprived of this protein, and found that its survival is drastically impaired in human blood. This underlies the important role that this antigens plays for the survival of the bacteria in the human host. By using this protein to bind to human factor H, meningococcus is able to mimic human cells and prevent the activation of the complement pathway. Is there a way, the researches asked, to prevent this binding to human factor H and prevent the bacteria from escaping the immune system? The answer is yes. By inducing antibodies against factor H binding proteins the infection can be controlled.

The second antigen is NHBA (Neisseira Heparin Binding Antigen), which binds heparin, and the third is NadA (Neisseria adhesion A) which is a key element for adhesion of the bacteria to

human cells. The researchers are now studying how the bacteria adapts to living in the human blood, and what changes in gene expression are induced by the condition (temperature, in particular) the bacterium finds in the host. It turns out, in particular, that fHbp is up-regulated when the bacteria are exposed grown in human blood. This means that in vitro studies may not efficiently estimate the role of these antigens in the pathogen's virulence and their contribution in immunogenicity.

Gut Immune Maturation Depends upon Colonization with a Host-Specific Microbiota

Dennis L. Kasper

Department of Microbiology and Immunobiology, Harvard Medical School

We may not always like them, but we definitely need them. It is well known that microbes living in our guts provide many beneficial effects, and this phenomenon extends to other animals as well. In fact, many plants and some insects are totally dependent on specific microbes with which they have co-evolved. Wondering whether the same might be true for mammals, Dennis Kasper evaluated changes occurring in the intestinal immune system when the microbes that usually inhabit the guts of mice are replaced with microbes from other mammals.

Dr. Kasper's group studied germ-free mice, which are born and raised in sterile conditions and have no microbes at all in their guts. The investigators colonized some of the mice with the contents of a mouse colon and colonized the rest with a human microbiota—i.e., bacteria taken from the human digestive tract. They found that the number of bacteria in the guts of mice in the two colonized groups was basically the same, but that the bacterial species present (especially those in the phylum Firmicutes) differed widely. More important, the number of cells of the immune system turned out to be different in the two groups. Mice colonized with human bacteria had lower levels of CD4+ and CD8+ T cells, few proliferating T cells, few dendritic cells, and low antimicrobial peptide expression. In other words, the latter mice had the same immunologic deficits as germ-free mice.

This result was surprising. In light of the substantial distance between humans and mice in evolutionary terms and in diet, the researchers next repeated exactly the same experiment in rats, which are very similar to mice in genetic terms and eat a nearly identical diet in research colonies. When germ-free mice were colonized with the contents of the rats' guts, the mix of bacterial species found in the rat microbiota was closer to that in humans than to that in mice. Moreover, even rat microbes did not complement the immune-system deficiencies in germ-free mice. Numbers of dendritic and immunoglobulin-producing cells were again considerably lower than in ""normal"" mice—i.e., those with their bacteria in the right place.

In order to double check, Kasper and his colleagues took mice colonized with a human microbiota and mice with the normal mouse microbiota and had them live together, so that they could exchange their bacteria and level out the differences. After four weeks, mice that had been colonized with a human microbiota had recovered most immune functions.

Cell counts in the gut are one thing, but the actual functionality of the immune system is another. What impact do these changes in the gut microbiota have on an animal's resistance to infectious diseases? To answer this question, Kasper and his group challenged the different populations of mice with pathogens, specifically with Salmonella bacteria. Whereas mice with their own microbiota in place proved highly resistant to infection, those with no gut bacteria and those colonized with microbes taken from humans were very sensitive to Salmonella. The latter groups became infected and soon had high numbers of bacteria disseminating in the spleen. Colonization of germ-free mice with some specific mouse-gut bacteria that appear particularly critical, such as segmented filamentous bacteria, made things a bit better but did not restore the immune system. Therefore, it is likely that several different microbial species are necessary for the establishment of a healthy immune system and that these bacteria are preferably normal residents in the microbiome of this mammalian species.

It certainly appears that mammals have coevolved with gut bacteria to the point at which some of these bacteria are critical for the development of the immune system. Is the same true for humans as well? And in this case, is city life, which minimizes the exposure of most of us to the microbes with which we have coevolved, damaging our immune system? Kasper suspects that this is the case and that the currently growing prevalence of autoimmune diseases such as asthma, multiple sclerosis, and inflammatory bowel disease may be, at least in part, the consequence of the increasing vulnerability of the coevolved human-microbe relationship.

Interleukin2 as a Novel Innate Cytokine

Paola Ricciardi-Castagnoli

*SIgN, A*STAR, Singapore*

Dendritic cells (DC) are among the first sentinels of the immune systems in mammals. They can be found on tissues that are most exposed to pathogens, such as the skin and the inner lining of the nose, stomach and intestines. Their job is to recognize harmful microbes, pick up their antigens (the molecules, typically on their outer surface, which can trigger the production of antibodies in the host's immune system), migrate in the lymphnodes and present antigens to the main players in the immune response: B cells (which produce antibodies) and T cells (which either kill infected cells directly or assist other cells in doing so), thus kick starting the adaptive immune response. Furthermore, dendritic cells also play a key role in inducing and maintaining immune tolerance to self-antigens. The recognition of their role in initiating the immune response earned the Canadian immunologist Ralph Steinman (who coined the very term ""dendritic cell"") the 2001 Nobel Prize for medicine and physiology. Although many studies have assessed the diverse functions of DCs, there are still many unanswered questions regarding the molecular machinery they use to do their job. This is the focus of the work of Paola Ricciardi-Castagnoli's group at the Singapore Immunology Network (SIgN), part of the Agency for Science, Technology and Research (A*STAR) in Singapore.

In particular, Ricciardi-Castagnoli's group has discovered that dendritic cells produce interleukin 2, a cytokine, which activate both NK (Natural Killer cells) and lymphocytes.

Long believed to belong exclusively to the adaptive immune response, IL-2 is now recognized to play a key role in regulating both innate and adaptive immunity. After discovering IL-2 production in DC in 2001, Ricciardi-Castagnoli has been investigating the signals that induce its transcription. Many different bacteria, fungi and parasites, including Schistosoma, and Leishmania induce a significant IL-2 production by DC.

Ricciardi-Castagnoli is now trying to work out how DC uses IL2 to regulate the immune reactions. She has shown that in order to activate the transcription of IL2, DC and their progenitors use a molecular pathway (i.e. a cascade of chemical events) which starts with a calcium flux, leading a protein called calcineurin to activate a family of transcription factors call NFAT (nuclear factor of activated T cells), which in turn lead to the production of IL-2. The next step will be to study the exact role this DC-derived IL-2 plays in living organisms in regulating the immune response. It seems that its main function is to activate regulatory T Cells (which ""self- check"" the immune system by suppressing immune responses of other cells) and CD8 T cells, which are a group of T cells tasked with killing infected and tumor cells. Ricciardi-Castagnoli's group is now working to verify this hypothesis.

The Gut Microbiota: In Sickness and in Health

Wendy S. Garrett

Harvard School of Public Health, Harvard Medical School, and Dana-Farber Cancer Institute.

The human colon is possibly the most densely populated microbial ecosystem on the planet, home to millions of bacteria of countless different species. In light of this, and considering the growing evidence that members of the bacterial world can contribute to tumors (HPV, which strongly associated with cervical cancer, is a typical example), it is surprising that there are relatively few studies on the possible links between gut bacteria and colorectal cancer. Wendy Garrett's lab at the Harvard School of Public Health is focused on understanding the contribution of gut microbes to health and disease with a focus on inflammatory bowel disease and colorectal cancer.

Two years ago, Garret's group had shown that certain bacteria that inhabit the intestine provide the environmental trigger that initiates and perpetuates chronic intestinal inflammation in individuals who are genetically susceptible to inflammatory bowel disease (IBD). Ibd is a devastating and debilitating chronic illness, and also one of the three highest risk factors for the development of colorectal cancer.

At the seminar, Garrett discussed recent studies of colorectal cancer microbiome and data from a collaboration between her lab and that of Matthew Meyerson (Dana-Farber Cancer Institute, Harvard Medical School, and the Broad Institute. Meyerson's lab has developed an informatics platform called PathSeq that allows researchers to go through vast amounts of genomic information coming from cancer genome studies (based on genomic sequencing of cancer tissues samples) and recover and analyze those sequences that belong to microbes.

In a publication led by the Meyerson lab, PathSeq helped identifying an association between fusobacteria and colorectal cancer. Tumor tissues and normal colon tissue from patients from Europe, Asia, and the U.S. were deeply sequenced and the sequences were subsequently analyzed using PathSeq ""What we saw was surprising"" Garrett recalls. Fusobacteria, which like to live our mouths, were clearly enriched in the tumors.. Could the presence of those bacteria be a hint, or even the cause, of a colorectal cancer? Tumor and normal colonic tissues from an additional 95 patients were used to check whether the association between tumor and bacteria still held on. It did, and further and more detailed analysis of the cancerous tissue reveled that fusobacteria are found deep in the tumor, and not simply on the surface or surrounding tissues.

The remaining question, though, is by what mechanism this happens. Garrett believes the answer must lie in the interaction of fusobacterium with the immune system and in its metabolism. Garrett's hypothesis is that fusobacteria might affect the immune system and favor the growth of tumors. Ongoing studies in the Garrett Lab are employing mouse models to understand how fusobacteria may cause colorectal tumors to develop and grow.

One thing, anyway, is already clear enough the microbial world may provide not only source of biomarkers for many disease but also new insight into pathogenesis of many diseases

ESX-1 Secreted Virulence Factors Control DC Response to Mtb Infection: Implication for Novel Vaccine Strategies

Eliana Coccia

Istituto Superiore di Sanità, Rome

Mankind has been trying to eradicate tuberculosis for a good part of the past century. And yet this disease keeps infecting one third of the worldwide human population, causing about 1.5 million deaths each year. One of the main reasons for this difficulty in controlling tuberculosis is the lack of an efficient vaccine. We do have one, called bacillus Calmette-Guerin (BCG) vaccine, but its efficacy varies greatly. It manages to limit the severe disease in children, but has little effect on transmission, and it becomes less efficient during adolescence. Clearly, if we are to win the fight with tuberculosis we need a new, improved vaccine, and the strategy to develop it is the focus of Eliana Coccia's work at Istituto Superiore di Sanità in Rome.

The key to a better vaccine may be in the understanding of how dendritic cells (a population of cells whose job is to recognize pathogens and present their antigens to the other cells of the immune system) react differently to the BCG vaccine and to the Mycobacterium tuberculosis (Mtb), the bacterium responsible for the infection. Dendritic cells modulate the immune response to the bacterium by presenting antigens to naïve T lymphocytes, as well as by promoting a selective recruitment of activated T and NK cells in the infected lung. It turns out dendritic cells infected with the vaccine do not fully mature, unlike those infected with clones of the actual bacterium, which may explain the partial efficacy of the immune response. Why does that happen? The attenuation of the vaccine strain BCG is largely due to the loss of a region of the genome called RD1 region, also known as the ESX-1 secretion system, which is responsible for the secretion of two proteins called EsxA (6-kDa early secreted antigenic target, ESAT-6) and EsxB (10-kDa culture filtrate protein, CFP-10), both key players in virulence and host-pathogen interaction. By infecting dendritic cells with Mtb and BCG recombinant strains expressing the wild type or the mutated/deleted version of RD1, the researchers understood that other, not yet identified molecules in the bacterium might cooperate with ESAT6 and CFP10 in regulating the functionality of dendritic cells.

Coccia and her group also investigated what happens in infected dendritic cells when it comes to autophagy, the process by which a cell digests parts of itself in order to remain healthy. This looks like a promising pathway for optimizing Mtb vaccines, and Coccia has shown that in human primary dendritic cells Mtb, but not the attenuated BCG version, inhibits the late steps of autophagy. For this block to happen, a functional ESX-1 secretion system is required. In addition, treatment with rapamycin (an inducer of the autophagic process restores autophagy in Mtb-infected dendritic cells, at the same time boosting the production of cytokines such as IFN-b and IL-12). At this point, dendritic cells are again able to start a robust immune response. These findings underscore a previously unrecognized link between Mtb virulence and autophagy inhibition and, together with the findings on maturation and cytokine expression, indicate that Mtb may control DC immunoregulatory functions through the ESX-1 secretion system.

Coccia's researches are (still in progress, but they do already show that dendritic cells might hold the key to develop a more effective tuberculosis vaccine, at least by being the ideal in vitro model for testing the properties of new vaccine candidates and anti-tuberculosis strategies.

Novel HIV Vaccine Strategies

Dan H. Barouch

Division of Vaccine Research, Beth Israel Deaconess Medical Center, Boston, MA, USA

AIDS is now a much different disease than it once was. In the developed world in particular, infections are decreasing, and AIDS has mostly turned into a chronic, controllable disease. On a global scale though, the epidemic is still a major concern. Though it seems to have stabilized with a 19% reduction in AIDS mortality and new infections between 2009 and 2011, there were still 33.3 million people living with HIV/AIDS in 2009. The total number of persons living with HIV/AIDS worldwide has increased, and less than one-third of people who need antiretroviral drugs have access to them. In 2009 there were 2.6 million new HIV infections and 1.8 million deaths due to AIDS.

Despite the excellent results obtained with antiretroviral therapy, a preventive vaccine still seems fundamental to stop the epidemics. And yet its development has hitherto eluded immunology. Three vaccine concepts have been tested over the last thirty years: one based on humoral immunity (the immune response mediated by antibodies), one on cellular immunity (which is mediated by lymphocytes) and the most recent one, which combined the two. None of them has delivered the expected results, and we clearly need something new. Dan Barouch's group at the Beth Israel Deaconess Medical Center is working to develop new strategies which may finally lead to a truly effective vaccine.

First of all, let's remember that a vaccine is made of two key parts. The first one is a vector, typically a deactivated live virus, which brings the vaccine into the organism and alerts the immune system, but has lost the ability to replicate and cause a real infection. The second part, which is artificially mounted on the vector, is the antigen, a selected part of the original virus's genetic material which is used to elicit an immune response, leading the immune system to produce specific antibodies which will permanently protect the organism from the infection.

A new generation HIV-1 vaccine should include two features: new vectors that avoid high levels of baseline antibodies to the vector (otherwise the immune system would have no time to get in contact with the ""real"" antigen and develop immunity), and that can be combined into an effective prime-boost regimen (the typical way a vaccine is used, with two doses at different times, and based on two different vectors); and antigens that elicit both humoral and cellular immunity and that optimize coverage despite the notoriously huge global diversity of HIV.

The first part of Barouch's work was, then, the search for suitable vectors. There are a handful of viruses which can be used, once deactivated, as a vaccine vector, most notably adenovirus such as Ad5, Ad26, Ad35, Ad48. By screening blood serum of 4000 individuals worldwide, Barocuh and his colleagues last year assessed how much antibodies for each virus population they had. A large part of the population, especially in South Africa, has a high content of antibodies for Ad5, making it less suitable. The most promising figures were those for Ad26 and Ad35.

The researchers then conducted a study on rhesus monkeys with various vectors expressing SIV (the monkey equivalent of the HIV virus) antigens. After trying Ad26, Ad35, and MVA Modified

Vaccinia Ankara (MVA), showed that Ad35/Ad26 as well as Ad26/MVA prime-boost regimens afford partial protection against both acquisition and infection.

The second part of the work is the antigen. The main problem preventing an effective HIV vaccine is the extreme genetic variability of the virus. Barouch and his groups have tried to circumvent the problem by designing ""mosaic"" antigens, computer-assembled genetic sequences that assemble fragments of natural proteins in the virus in order to make them, so to speak, more representative of the global virus population. By applying this method to the three key genes which are responsible for the virus's structural replication (called Gag, Pol and Env) they were able to obtain a stronger and more durable immune response in rhesus monkeys, compared to what can be obtained with natural sequences. The future strategy followed by Barouch's group will be made of two steps: first, saveloy “prototype” novel Ad vectors expressing a single test antigen which seems to be particularly critical for producing an immune response (called VRC EnvA) for a rapid assessment of vector safety and immunogenicity in humans. Second, develop “complete” vaccine products involving optimal heterologous prime-boost regimens expressing multiple HIV-1 antigens (mosaic Gag/Pol/Env) for clinical development.

Immune Responses to Herpes Simplex Virus: From Sensing of Foreign Viral DNA to Vaccines

David M. Knipe

Harvard Medical School

The Siena workshop could not neglect one of the best known and most common viruses affecting humans: herpes simplex virus, which infects a large share of the human population, typically remaining latent in the cells of the nervous system and reappearing every now and then when it migrates to the skin causing cold sores or genital herpes.

The family includes two related viruses: HSV-1 rarely cause serious problems, unless it infects the cornea, where it can lead to blindness, or unless it colonizes the central nervous system, where it becomes life threatening, causing about 1000 cases of lethal encephalitis a year.

The second type, HSV-2, is less prevalent in the USA and more widespread in African countries. It is life threatening in neonates and causes infections in AIDS patients. In Subsaharian Africa there is a coepidemic of genital herpes and HIV, and it is clear that general herpes increases the risk of HIV infection up to three fold. A vaccine would definitely be helpful, and three attempts to develop one have been done over thirty years, but have not led to a licensed vaccine.

David Knipe's group is exploring new strategies, starting from the biology of the virus. The HSV virus infects the mucosal surface, then replicates, and before the immune system can clear it, the virus enters the axons of sensory neurons and travels to the ganglia, where it remains and the viral DNA establishes late infection. Subsequently the activation of neurons can lead to the sporadic infection. The virus binds to the cell, enters the nucleus, releases the DNA, which is transcribed to RNA, leading to the production of proteins which in turn promote the replication of the viral DNA itself.

The group has been testing a new approach, a genetically engineered virus with mutations in DNA replication proteins. Called dl5-29, this mutant virus strain has deletions in the U(L)5 and U(L)29 genes. It expresses a large number of antigens in the cell, but cannot replicate. In guinea pigs and mice, the new vaccine candidate has proved to work, protecting against challenge with wild-type HSV-2 and against ocular disease caused by HSV-1, and reducing both acute and late infections. A phase I trial will start next year.

Knipe and his colleagues are now trying to improve their vaccine by knocking out the functions which normally allow the virus to escape the immune response. This involves knocking out some proteins which the virus uses to block among others, the reaction of dendritic cells. Fortuitously, though, it seems that the current dl5-29 formulation may already come equipped with reduced capacity to turn off these immune invasive function, and Knipe is working to clarify by what mechanism this happens.

Human CMV Drives Rapid NK Cell Maturation after HSCT

Moretta Alessandro and Della Chiesa Mariella

Dipartimento di Medicina Sperimentale,University of Genova ,Genova, Italy

Natural Killer (NK) cells are the first line of defense of the innate immune system. They respond quickly to infection and tumor formation, typically attacking and killing affected cells in a few days. They also play a crucial role in hematopoietic stem cell transplantation (the transplantation of cells derived from the bone marrow to treat myeloma or leukemia), because they are the first cells to recover after the intervention. One of the world's leading experts on Natural Killer cells, Alessandro Moretta from the University of Genoa, told participants in Siena two stories that show how NK cells can affect the outcome of a bone marrow transplantation in different patients.

The first story involves human Cytomegalovirus (CMV): a virus, part of the Herpes family, which affects a large part of the population and typically remains latent in the organism. This virus is known to affect the immune system, and Moretta investigated what effect CMV has on the differentiation of NK cells in individuals undergoing bone marrow transplantation.

NK cells derive from the bone marrow and are released as immature NK cells. At this stage, they typically express high levels of CD56, a protein which favors adhesion between cells (they are thus called CD56 bright) and NKG2A, a receptor which helps these cells between pathogens and the cells of its own organism. Moreover they express a protein called CCR7 which causes them to migrate towards lymph nodes. Once they get there, they complete their maturation. The most evident change they undergo is that they reduce the expression of CD56 thus becoming CD56 dull. So, by observing the expression of CD56 it is easy to differentiate mature (CD56 dull) and immature (CD56 bright) NK cells.

The immature NK cells do not express Killer-cell immunoglobulin-like receptors (KIRs), a family of receptors specific for HLA class I molecules that will later regulate their killing function. But once they reach the lymphnode, the phenotype changes abruptly. CCR7 is lost completely, the cells reduce both CD56 and NKG2A and start to express KIRs as well as another protein, CD57.

So, in a normal individual, mature NK cells will be CD56 dull, while KIR will be found on a defined subset. Moretta and his group studied the population of NK cells after transplantation, comparing the situation in individuals with or without a latent CMV infection. Whereas after one month there was no great difference, six months after the transplantation the picture changed dramatically. In 'normal' individuals (i.e. with no CMV infection) there are many immature NK cells, while in those reactivating CMV these cells have almost completely disappeared and are replaced by mature NK cells. Most important is the increase in KIR positive cells as compared to individuals with no CMV.

Moretta says he and his team do not know exactly how and where the CMV infection promotes NK cells maturation. It may happen in the bone marrow, in the peripheral tissues, or in lymph nodes. "We favor the interpretation involving lymphnodes but is an open issue" he says. But the importance of this finding is related to the fact that KIR-positive NK cells are the only ones that can mediate alloreactivity. And - as shown by Lorenzo Moretta's work elsewhere in this report - alloreactive NK cells can be useful in leukemia patients to prevent tumor relapses and graft-versus-host disease (the situation where immune cells from the donor attack the recipient's tissue). Thus

the acceleration of NK cell maturation induced by CMV appears of crucial relevance for the generation of KIR+ alloreactive NK cells early after transplantation.

The second story relates to NK cells recruited from the blood into inflamed tissues in response to pathogen invasion. At these sites NK cells become activated and acquire the capability to mediate the so called editing process of myeloid dendritic cells. During this interaction, not only dendritic cells but also NK cells acquire CCR7, but as said above, most NK mature cells are CCR7 negative. How do they manage to acquire it a second time?

It turns out this is due to two different mechanism. First, they uptake it from surrounding cells expressing this receptor, for example myeloid dendritic cells. But they also use another mechanism which is based on a cytokine, IL 18, which in turn can induce the expression of CCR7.

But what mechanism can be responsible for IL 18 release? Dendritic cells do not produce it. The most obvious candidates are macrophages, large cells whose function is to engulf and digest pathogens and cellular debris. The researchers analyzed the interaction between NK cells and different types of polarized (i.e. activated towards specific functions) and unpolarized and non-activmacrophages, showing that a real ""symbiotic"" process is at work. During this process unpolarized macrophages that express IL18 on the cell membrane are induced to release this cytokine when undergoing polarization towards M1 in response to lipopolisaccaride. Moreover subsets of M1 polarized macrophages acquire surface CCR7. IL 18 induces not only production of interferon gamma in NK cells but also their reacquisition of CCR7, so that they can migrate to lymphnodes. This process is crucial also for macrophages themselves, because the interferon gamma produced by NK cells is crucial for their activation.

Finding Genes and Networks of the Immune System

Nir Hacohen

Massachusetts General Hospital

The immune system is a really complex biological system that can sense infections, wounds, tumors, allergens, but what is it? Is it like an ecological system that is molded by predator-and-prey relations? Or is it like a thermostat sensing a changing environment and trying to maintain the body's equilibrium? Answering those questions is the work of Nir Hacohen at the Broad Institute and Massachusetts General Hospital.

One way in which these problems are being addressed at the Broad Institute is by using the tools that build on the Human Genome Project, enabling a comprehensive analysis of the immune response for the first time in history.

A few years ago Hacohen's team, in collaboration with other international research groups, realized a powerful instrument for mammalian genetic screens.

Its name is RNA interference (RNAi), and consists of an extensive "library" of 320.000 RNA molecules that can switch off genes individually. This platform allows scientists to identify the genes underlying disease processes and to test their role in fending off infectious foreign material.

Hacohen is focused on applying the library to the study of immunology and infection, with the ultimate goal of creating a comprehensive genetic map of the innate pathogen sensory system.

In particular, he started a cumulative study of pathogen sensing to create a multi-layered network model. The starting point is the innate immune system as opposed to the adaptive immune system: the former is the body's rapid and non-specific reaction to almost any acute infection, while the latter is the body's method for preparing for long-term attacks by specific pathogens each time they are encountered.

All mammals have them both, and they are both essential. Yet the innate system does have a unique sensing mechanism: dendritic cells, which identify pathogens and generate responses.

Studying this cell type in humans and mice, Hacohen observed that it presented an ordered cascade of processes during maturation: pathogen recognition, engulfment and destruction; antigen processing and presentation; production of cytokine and chemokine (signaling molecules used in intercellular communication); migration to lymph nodes (immune cells acting as filters or traps for foreign particles); and finally, engagement of CD4 and CD8 T cells (type of white blood cells that play an important role in the immune system).

Dissecting the Human T Cell Response to Microbes

Federica Sallusto

Institute for Research in Biomedicine, Bellinzona

Our immune system has a long-lasting memory. Most of the times, when a pathogen first infects the body, nothing happens before several days. But when the same pathogen is encountered a second time, the response is rapid and vigorous.

This phenomenon, known as immunological memory, is the basis for vaccination. It is due to the activation of white blood cells playing a central role in immune defense, B and T cells. The latter are particularly specialized: they carry immunological cell-mediated immunity, for which the protective function of immunization is associated with cells rather than antibodies.

Federica Sallusto, from the Institute for Research in Biomedicine, Bellinzona, Switzerland, is one of the leading immunologists of our time. Over the years, she has given outstanding contributions to the current understanding of human T cell responses and their polarization toward functionally distinct subsets.

It's no wonder that such an important component of the immune system has to be so specialized; but understanding how this diversity is generated is crucial to identify all the steps needed for the immune response.

Sallusto and her team carried on several research studies isolating different subsets of T cells and studying them in vitro. The goal was building up the link between the differentiation of T cells and the ways they are involved in the immune response to pathogens. A fundamental aspect, which has been so far underestimated.

In order to reach this aim, Sallusto's Lab introduced novel high-throughput technologies to dissect antigen-specific immune responses, by using specially developed human "T cell libraries". These tools allowed the isolation of specific T cells subsets from peripheral blood, like Th1, Th2, Th17 and Th22.

T cell libraries from these subsets were generated and simultaneously interrogated for reactivity against a panel of antigens from viruses (e.g. Cytomegalovirus, Hepatitis B, Influenza virus), bacteria (e.g. Mycobacterium tuberculosis Staphylococcus aureus) and fungi (e.g. Candida albicans). This analysis led to gain important information on the distribution, frequency and class of the human T cell response to different pathogens.

This approach, exploiting the highly differentiation of T cells, can be useful for the evaluation of vaccine candidates, an even for the development of new cellular immunotherapies.

How HIV Evades Innate Immunity

Judy Lieberman

Immune Disease Institute and Program in Cellular and Molecular Medicine, Children's Hospital Boston, and Department of Pediatrics, Harvard Medical School, Boston MA US

One of the reasons HIV is able to establish a foothold in a new host is that it somehow manages to elude one of the most important lines of defense in our innate immune system: the sensors contained in the cytosol (the liquid inside cells) that detect foreign DNA and RNA. Judy Lieberman is trying to understand how it manages to do this. The main character here is Trex 1, a nuclease (i.e. an enzyme that breaks the bonds between subunits of nucleic acids) discovered about a decade ago. Although it digests DNA, which is located in the nucleus of cells, most of TREX1 is unexpectedly found in the cytosol. Several studies in knockout mice tried to understand what it actually does. Mice lacking the gene for Trex1 die of inflammatory heart disease. In humans, TREX1 mutations are linked to inflammatory and autoimmune syndromes such as Aicardi-Goutiere's syndrome, systemic lupus erythematosis and chilblain lupus, and retinal vasculopathy with cerebral leukodystrophy. These diseases may be linked to elevated interferon production. The researchers suspected that one job of TREX1 is to digest DNA in the cytosol. Cytosolic DNA can come from endogenous retroelements or from infection with DNA viruses or retroviruses. If it is not digested, it triggers innate immune sensors of cytosolic DNA that activate expression of interferons, a potent group of antiviral molecules.

When cells in which the Trex1 gene had been eliminated are exposed to HIV, DNA from the virus builds up in their cytosol, triggering an interferon response, which would normally be absent in wild-type cells, and the interferons in turn inhibit HIV replication.

At this point Lieberman wanted to figure out what pathway, i.e. what biochemical sequence of events, triggers interferon production when HIV DNA builds up in the cell. They performed in vitro studies knocking down all the known sensors for DNA and RNA to see what happened, but none of them was responsible for interferon production. The only sure thing is that the process depends on three signaling molecules called Irf3, Sting, and Tbk1, known to be involved in activating interferon expression.

So the obvious question is: if we could trigger interferon production by silencing Trex 1 at the point of infection, would that prevent transmission? Although this is an attractive idea, it could turn out to be a double-edged sword. In addition to producing an antiviral immune response, it would recruit more immune cells to the infection site and provide more substrate for the virus to spread.

The best way to test the idea was to silence Trex1 locally in the genital tract and see what affect that maneuver had on sexual transmission of HIV. The researchers used a technique they developed to silence selected genes in vivo in the immune cells that HIV infects with small interfering RNA (siRNA), short sequences of RNA which can selectively block the expression of targeted genes. They were thus able to prove in humanized mice (mice carrying human tissues that make them susceptible to HIV infection) whose Trex genes had been silenced, that HIV infection was indeed inhibited. The Trex1 gene thus looks like it might be a promising target for new HIV preventive therapies.

Glossary

Adaptive immune system: the body's preparation for long-term attacks by specific pathogens each time they are encountered. It is composed of highly specialized, systemic cells and processes that eliminate or prevent pathogenic growth.

Antibody: large Y-shaped protein produced by B cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses.

Antigen: any substance that evokes the production of one or more antibodies. It may be a foreign **substance** from the environment such as chemicals, bacteria, viruses, or pollen; it may also be formed within the body, as with bacterial toxins or tissue cells.

B cells: a group of white blood cells known as lymphocytes, playing a crucial role in the humoral immunity branch of the adaptive immune system, the one giving our body the ability to recognize and remember specific pathogens each time they are encountered.

CAPS: Cryopyrin-Associated Periodic Syndromes, a group of rare, inherited, autoinflammatory diseases characterized by severe inflammatory symptoms such as fever, rash, arthritis, growth delay, neurosensorial defects.

CMV: cytomegalovirus, a viral genus of the viral family known as Herpesviridae or herpesviruses.

Cytosol: also called intracellular fluid or cytoplasmic matrix, is the liquid found inside cells.

Dendritic cells: immune cells forming part of the mammalian immune system, with the main function of processing antigen material and presenting it on the surface to other cells of the immune system.

Exotoxin A: also called Pseudomonas Exotoxin, is an exotoxin (toxin secreted by bacteria) produced by Pseudomonas aeruginosa. It inhibits elongation factor-2, a protein that in humans is encoded by the EEF2 gene and that is an essential factor for protein synthesis.

IECs: intestinal epithelial cells, the cell boundary between the external environment and tissues of the gastrointestinal tract.

IL-1β: Interleukin-1 beta, also known as catabolin, is a cytokine protein that in humans is encoded by the IL1B gene.

IL-2: Interleukin-2, an interleukin, which is a type of cytokine signalling molecule in the immune system.

Innate immune system: the body's rapid defense from almost any acute infection. It comprises the cells and mechanisms that defend the host from infection by other organisms in a non-specific manner.

IPEX Syndrome: Immunodysregulation Polyendocrinopathy Enteropathy X-linked Syndrome, a rare disease linked to the mutation of the protein FOXP3, partially responsible of the immune system responses.

IRF3: Interferon regulatory factor 3, an interferon regulatory factor (proteins regulating transcription of interferons).

Lymph nodes: immune cells acting as filters or traps for foreign particles.

Lymphocyte: a type of white blood cell in the vertebrate immune system.

MAMPs: Microbe-Associated Molecular Patterns, molecules associated with groups of microbes that are recognized by cells of the innate immune system.

NK cells: natural killer cells, a type of cytotoxic (toxic to cells) lymphocyte critical to the innate immune system.

NS1: non-structural protein 1, encoded by the influenza A virus, is an RNA-binding protein that is required for virus replication.

Nuclease: an enzyme capable of cleaving the phosphodiester bonds between the nucleotide subunits of nucleic acids.

ORFs: open reading frames, a way of dividing the sequence of nucleotides to identify candidate protein coding regions in a DNA sequence. It is the part of a reading frame that contains no stop codons.

RLRs: RIG-I-like receptors, a type of intracellular pattern recognition receptor involved in the recognition of viruses by the innate immune system.

siRNA: small interfering RNA, also known as short interfering RNA or silencing RNA, is a class of double-stranded RNA molecules, 20-25 nucleotides in length.

T cells: a group of white blood cells known as lymphocytes, playing a central role in cell-mediated immunity, important in defense against pathogens, autoimmune diseases, some acquired allergies, and other immune reactions.

TREX1: Three prime repair exonuclease 1, an enzyme that in humans is encoded by the TREX1 gene.

TRIM25: Tripartite motif-containing protein 25, a protein that in humans is encoded by the TRIM25 gene.