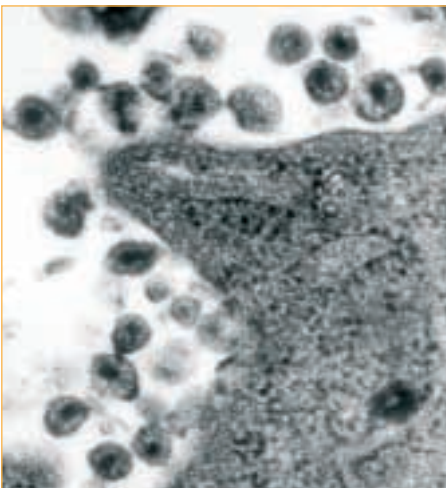




Beating Them with Their Own Weapons New HIV Vaccines Undergoing Clinical Trials

The development of a vaccine stimulating the immune system to fight HIV is one of the greatest challenges in AIDS research. Vaccine research is faced with the big task of developing a therapeutic vaccine against an already existing infection. At the GSF Institute of Molecular Virology scientists have now achieved some first success with a vaccine on the basis of a genetically modified vaccinia virus, which is now going to be combined with another vaccine to produce a powerful combination. Using such combination vaccines, scientists hope to one day be able to protect healthy people from infection.

Despite all information campaigns AIDS is advancing throughout the world. It is mainly in southern Africa that the disease has taken disastrous dimensions, but in numerous other countries the number of new infections is also rising rapidly. Although there are meanwhile highly effective antiviral therapies, these are unaffordable, particularly in third-world countries – only five per cent of all those who are infected worldwide have access to these drugs. Apart from that modern therapies (HAART – highly active anti-viral therapy) keep the viral load low for a long time and thereby prevent an outbreak of the disease, but they cannot remove the virus from the body altogether.

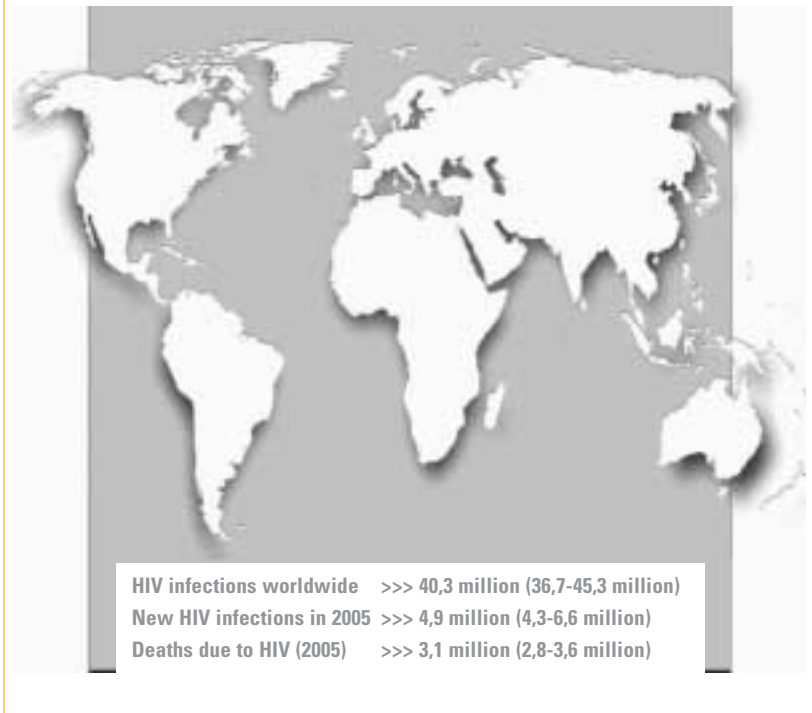


Scanning electron microscopy has visualised HIV and the cells of the immune system. Many viral particles bud from the surface of an infected CD4 cell. Measuring the amount of virus in the plasma enables physicians to detect the levels of a patient's viral load.

Vector Vaccine with Special Design

“The purpose of vaccinating must be to stimulate the immune system of patients who are already infected with HIV, so that the outbreak of AIDS will be delayed or even prevented,” explains Professor Volker Erfle, the recently retired Director of the GSF Institute of Molecular Virology. The scientists from the Institute first developed a vector vaccine on the basis of harmless genetically modified MVA viruses (modified vaccinia virus

HIV infection rates in 2005



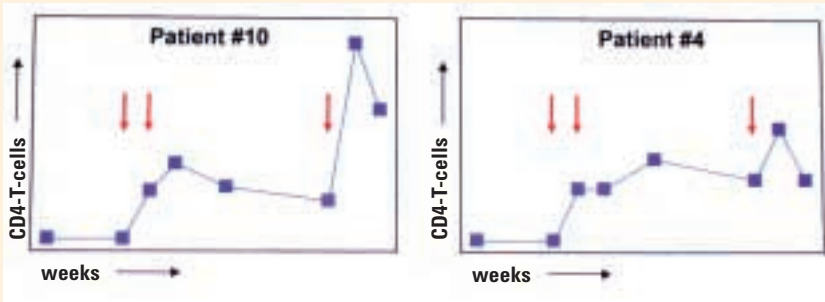
In 2005 alone 4.9 million people were infected with HIV worldwide.

Ankara), into which the design for the HIV protein Nef was introduced. Nef was chosen, because it is of decisive significance in the life cycle of the virus: Nef is produced by infected cells soon after the infection and causes an effective reproduction of the virus. In the absence of Nef AIDS does not break out. If the vaccination works, it will beat the pathogen with its own weapons: the inoculated vectors invade body cells and stimulate them to produce Nef, so that the immune response against Nef is stimulated and Nef is deactivated. In this process both antibodies against Nef are produced (= humoral defense) and specific defense cells are activated, which destroy the infected cells (= cellular defense). “Our idea was to look for a therapeutic vaccination which stimulates the immune response, so that the number of virus-specific CD4-positive T-cells remains high,” explains Dr. Antonio Cosma from the GSF Institute of Molecular Virology.

As a first practical test Erfle and Professor Frank Goebel, the head of the outpatient AIDS department at the Munich Innenstadt-Kliniken, and scientists from the Munich Klinikum rechts der Isar, conducted a clinical phase I study on vaccination with MVA-Nef.

Helper Cells at Work

Increase in HIV-Nef-specific CD4-T-cells after immunization (↓) with a vaccinia virus (MVA)-based HIV-Nef vaccine



Defeating the pathogen with its own weapons: after immunization with a vaccinia virus (MVA)-based HIV-Nef vaccine specific CD4 defense cells are activated, which help to destroy infected cells.

CD4-positive T-cells are so-called helper cells, which stimulate the immune system and are of decisive significance to the development of protective immune responses. In infections the affected tissue produces certain antigens which are recognized by T-cells. The number of the T-cells reacting specifically to the respective antigen is, therefore, good evidence of the

defense capability of the immune system. HIV attacks these cells, this is why their number usually drops after an infection. So-called LNTP patients ("long-term non-progressors"), who do not have an outbreak of AIDS even without therapy, although some of them have been infected for more than 20 years, show that the

immune system can basically control HIV on its own. They manage to hold the virus at bay, because their immune system has a different response to an HIV infection: normally the number of CD4 cells rises after the infection, but then drops to very low values. In contrast to this, the number of the CD4-positive T-cells remains constantly elevated in LNTP patients.



With the Vector Group Prof. Dr. Volker Erfle, former Director of the GSF Institute of Molecular Virology, (right) developed a therapeutic vaccine against HIV. It stimulates the immune response, so that the number of the virus-specific CD4-positive cells remains high and infected cells are destroyed. Dr. Antonio Cosma (left) conducts the immune monitoring in the clinical studies.

The result was encouraging even after just one immunization: in four out of the ten patients vaccinated the number of Nef-specific CD4 cells increased. "This cell type had not been identified in any of the patients before, so that a clear immune reaction to the target structure HIV-Nef was seen," Cosma explains. All subjects had been infected with HIV for some time and were undergoing treatment with HAART, which was at first continued during the clinical study. After the vaccination seven of the ten patients agreed to stop the antiviral therapy. The number of HI viruses increased following this, but the immune response to Nef also increased – evidence that the patient benefits from the vaccination. Nevertheless six patients had to resume drug therapy after several weeks.

Practical Vaccine Test

The patient with the best response to the vaccination has been able to control the virus himself for nearly three years. Not only is the number of CD4 cells high and stable in this patient, he also shows a strong CD8 immune response. CD8-T-cells are cytotoxic cells

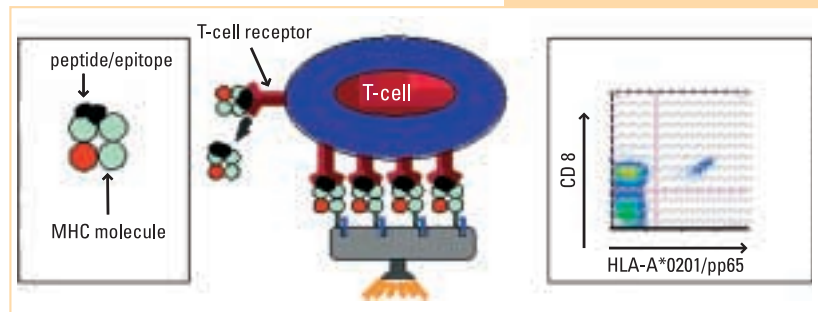
which recognize and destroy infected cells. CD4-T-cells produce growth and signal factors which cause the production and maintenance of CD8-T-cells. For successful immune defense there must be sufficient quantities of both cell types. "This patient is, of course, very interesting for us, because if we find out why his immune response is so strong, we might be also able to help other patients more," Cosma explains.

Keeping an Eye on Immune Response

Since there is often little change in the clinical condition of vaccinated patients, it is important to observe the condition of the immune system by good immune monitoring. To assess the success of the vaccination and find markers for the immune response, the scientists developed new methods which allow a deeper insight into immunological processes. "Using new methods of flow cytometry, for example, we can determine the phenotype of individual T-cells and define exactly which markers express these cells – up to 13 markers at the same time," Cosma explains. Thus, the scientists can analyze how T-cells react to the vaccination and characterize the immune response of individual patients. "In order to find markers for a particularly good immune response, we want to find out, for example, how the immune response of the patient with the best response to the HIV-Nef vaccination differs from that of other patients," says Cosma.

Better Characterization of T-Cells

An essential breakthrough for the monitoring of immune therapies was the establishment of the MHC tetramer technology by the Clinical Cooperation Group "Antigen-Specific Immunotherapy," headed by Prof. Dr. Dirk Busch. This method for the characterization of antigen-specific T-cell populations uses so-called MHC restriction: T-cells only recognize antigens, if they are presented by an MHC molecule. Individual MHC antigen complexes are, however, only weakly bound and dissociate quickly. Therefore, the scientists



crosslink several complexes with each other, so that structures with more stable binding are produced. As a rule four complexes are combined to so-called tetramers. Tetramers bound to T-cells, in turn, can be made directly visible using fluorescence dye. "Tetramer technology is a very useful instrument for finding virus-specific T-cells, which will then be further examined," Busch says.

Hopes for Combination Vaccine

One problem of all HIV vaccines developed so far is that the virus changes: it mutates very fast and even minor changes in its surface proteins may mean that it is no longer recognized by T-cells. In order to obtain an even

Prof. Dr. Dirk Busch achieved a substantial breakthrough for the direct study of antigen-specific T-cells with his Clinical Cooperation Group "Antigen-Specific Immunotherapy": with the MHC-multimer technology which he developed it is now possible to make epitope-specific T-cells visible and to isolate them with high purity.

Europe-Wide Search for Therapeutic Vaccine

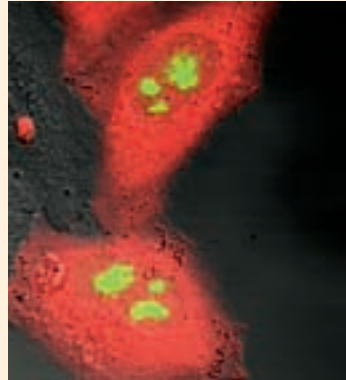
The vaccination studies have been part of European cooperations. In order to even better coordinate and accelerate the development of a new combination vaccine for HIV, all existing and planned activities throughout Europe have been pooled in one big joint project. Within the framework of AVIP ("AIDS Vaccine Integrated Project") 15 different working groups and institutions, including a working group at the GSF Institute of Molecular Virology, want to develop four new vaccines against HIV and test them on healthy test subjects in clinical phase I studies. All vaccines have the same combination of regulatory and structural HIV proteins. In 2009, when the five-year funding period ends, AVIP wants to present a vaccine which will be suitable for use as a therapeutic vaccine in clinical phase II and III studies on HIV-infected patients. For this purpose the AVIP consortium has more than Euro 20 million at its disposal, half of which comes from the 6th EU Framework Programme.



In the framework of the European large-scale project AVIP (AIDS Vaccine Integrated Project) scientists from the GSF Institute of Molecular Virology work on the development of a new combination vaccine. It should be used as a therapeutic vaccine for infected patients, but one day also protect healthy people from HIV infection.

Patent for HIV Diagnosis at an Early Stage

When HIV vaccines are tested on infected patients, it is particularly important to keep an eye on the course of the HIV infection. This is done by regularly measuring the virus load in the blood, in which the genomes of the HI viruses in the plasma are quantified. This method, however, can only detect free virus, the viruses "hidden" in cells are not found. Therefore, the working group of Dr. Ruth Brack-Werner, the new Provisional Director of the GSF Institute of Molecular Virology, developed and patented a method to show infected cells in the blood of HIV patients. For this purpose the patients' cells are isolated and treated with genetic engineering methods. As a consequence of this treatment infected cells in which the early regulatory proteins of HIV are active, stain red and can, therefore, be quantified," explains Brack-Werner, "this will help us to detect and document the effect of HIV-inhibiting substances or vaccines even earlier and more accurately."



With a process newly developed at the GSF and already patented, the activity (red) of early regulatory proteins of HIV can be quantified. This way Dr. Ruth Brack-Werner, new Provisional Director of the GSF Institute of Molecular Virology, wants to document the effect of HIV vaccines even earlier and more accurately."

more effective vaccine for the future, the scientists, therefore, plan to combine different HIV vaccines which attack the virus in different ways: some vaccines contain structural virus components, others act through regulatory proteins which control the reproductive cycle of the virus or the expression of its genes. If the new vaccine contains both structural and regulatory components, it will offer the immune system a broad range of points of attack, since the body builds up an immune defense against all injected components.

The GSF scientists will test the combination of MVA-Nef with another vaccine which confronts the immune system with the HIV envelope protein Env in a new clinical study as soon as in 2006. "This way we activate the immune system on the one hand via Nef to develop cellular defense mechanisms and on the other hand we increase antibody production via the Env protein, because the envelope protein is expressed on the surface and is, therefore, a good target for specific anti-

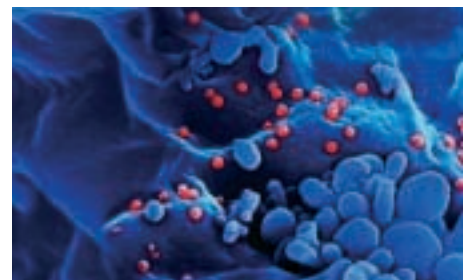
bodies," Cosma explains. In cooperation with Professor Goebel the clinical study will start out with 50 subjects who will be divided up into small groups, in order to be able to investigate all possible combinations of the vaccines. "If both are injected together, one vaccine tends to be dominating, while the other becomes ineffective," Cosma regrets, "therefore, it is better to administer the different vaccines one after the other: the next vaccination follows, when there has been a response to one vaccine."

The new combination vaccination will first be tested on healthy subjects. While MVA-Nef was intended to be used as a therapeutic vaccine for those who are already infected with HIV, the scientists hope to be able one day to use the new combination vaccinations to protect healthy people from infection. Whether such effective vaccine protection against HIV will actually be developed soon, is as yet uncertain. But even a vaccine which activates the immune system to such an extent that the risk of infection is reduced would be great help in the fight against the AIDS pandemic – mainly in third-world countries, where drug treatment for HIV is usually no option for most of the infected patients for financial and logistics reasons.



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At the new S3 laboratory established at the GSF Institute of Molecular Virology laboratory director Dr. Andrea Kleinschmidt is very strict about compliance with safety standards in the work with HI viruses.