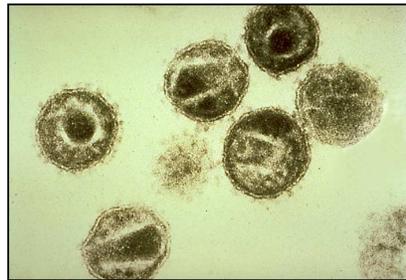


## HIV: « The Defensins strike back »



There is definitely no justice in this world. Even when faced with HIV, the virus that is responsible for AIDS, not everyone is equal. Indeed, a very small proportion of the population seems to be resistant to the infection and will undoubtedly never develop the disease. Three proteins, known as defensins, may be the reason for this inequity. And this apparent injustice could one day be the basis for developing a really effective treatment to fight the plague.

At first sight, the prostitutes of Marengo, a miserable shantytown on the outskirts of Nairobi, seem no different to other prostitutes working in other shantytowns around the world. Yet a number of these Marengo women have been part of an intense study since the early 1990s and may provide a solution, from the depths of their misery, for a treatment against AIDS. HIV appeared for the first time in Kenya in the beginning of the 1980s, and since then, the vast majority of the prostitutes of the region have been infected with the virus. However, Dr. Plummer, a Canadian scientist from the University of Manitoba, has shown that about 5% of these women are resistant to the infection, despite their behaving no differently from other women who have contracted AIDS, and who also suffer from other sexually transmitted diseases. Further studies in the United States revealed that between 1-2% of the HIV positive population, known as "long-term nonprogressors", do not develop the disease since the amount of the virus in their blood remains low. Scores of scientists tried to understand what distinguished these people from the rest of the HIV positive population. It was suggested that the cells of their immune system secreted a factor that stopped the virus, thereby giving them a kind of

natural immunity. After more than 10 years of research, a team from the Aaron Diamond AIDS Research Center of New York claims to have finally identified this elusive factor. However, all researchers have not accepted the finding unanimously.

### *The squatters of living matter*

A virus is nothing more than a piece of DNA or RNA protected by a couple of proteins. Alone it can do nothing; least of all replicate. But like a biological squatter, it introduces itself into a cell and then uses the cell's machinery to produce copies of itself. The normal consequence of viral infection is that the infected cell dies and liberates the deadly offspring, which then infect other cells. Each virus has a preferred cell type and will not attack other types of cells. Normally, after a certain period of time, our immune system - which is responsible for defending our body against such attacks - succeeds in destroying these unwanted intruders. Unfortunately, the problem with HIV is that the cells it prefers are the cells that are normally meant to protect us - the CD4 T-lymphocytes.

### ***The immune system: close quarters control***

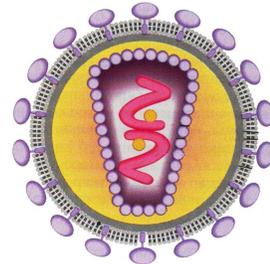
Made up of a complex network of organs and cells, our immune system protects us from invading bacteria, viruses, parasites and fungi. How? Each of these invading microorganisms has at least one unique and specific chemical component. This component is known as the antigen, a sort of label that marks the organism as being foreign to our body. It is during our embryonic development that our immune system learns to differentiate between our own antigens (self) and foreign antigens (non-self).

As soon as an antigen is recognized as foreign, our organism deploys a number of defense mechanisms, using a special type of cell: the white blood cells. For a start, the B-lymphocytes - a type of white cell - produce antibodies. These Y-shaped proteins circulate freely in the blood and attach themselves to the surface of the foreign cells, thereby marking the latter distinctively for the "cleaner" cells - the macrophages - which will subsequently destroy them. Then the CD4 and CD8 T lymphocytes make an appearance; so named because they bear a CD4 or CD8 protein on their surface, respectively. The CD8 proteins function as 'receptors' which recognize 'labels' on the intruders. As each lymphocyte has only one type of "receptor", it can only detect one specific intruder. However, with over 100 billion different lymphocytes, each one of us can virtually detect the same number of enemy intruders!! Once a foreign body is detected, the CD4 lymphocytes send a chemical message both to the B-lymphocytes, instructing them to produce antibodies which will neutralize the intruders that are circulating in the bloodstream, as well as to the CD8 T-lymphocytes to kill the infected cells. The AIDS virus essentially infects and destroys the CD4 T-lymphocytes.

### ***How does HIV replicate?***

Under the electron microscope, HIV looks like a lychee. The protuberances on its external shell are actually proteins known as gp120, which play a crucial role in the infection. Inside the shell resides the genetic material of the virus (a piece of RNA containing only 9 genes) and several proteins. The HIV virus loses its shell as soon as it has infected a cell, and by way of a viral enzyme, a DNA copy is produced from the viral RNA. This is the reason why the HIV is known as a retrovirus. The viral DNA is then integrated into the DNA of the infected cell. In this way, HIV remains hidden, literally, without producing any new viruses for a certain time. One day though, this happy symbiotic relationship comes to an end and the virus activates itself. As the cell no longer knows the difference between its genetic material and that of the virus, it starts producing copies of the viral

RNA and viral proteins which are then assembled in the cell. A number of viral proteins even order the cell to produce new viruses to the detriment of proteins normally produced by the cell. In effect, the sum of all these events eventually leads to the death of the CD4 lymphocyte.



**Fig.1 HIV section**

The virus fixes to the cell's surface by creating a link between its gp120 surface protein and the CD4 protein on the lymphocyte, which acts as an HIV receptor. In fact, the gp120 protein acts like a key. But in order to open the cell's door, the key also needs to attach to a second lock, known as the co-receptor. Two co-receptors, each recognized by a different type of HIV, were discovered in 1996. Although all HIVs look the same under the microscope, there is not "one" HIV but "several" HIVs. Indeed, after isolating a first virus, named HIV-1 in 1983, Prof. Montagnier of the Pasteur Institute and his team isolated a second virus, HIV-2, in 1986. The genetic material between the two differed by over 50%! Within these two groups, there were a multitude of different viruses which were sub-divided into groups and sub-groups, based on their genetic differences. These different HIV types or mutations were a result of errors caused by the viral enzyme whilst copying the RNA into DNA. Hence after several months, and in the same HIV positive individual, the viruses present in the blood are no longer the same as those that caused the infection!! And it is mainly at the gp120 level, i.e. the proteins found on the shell surface, that these mutations take place. Such mutations allow the virus to escape detection by the immune system since the antibodies that recognize the original viral proteins are no longer effective. In other words, they no longer recognize the HIV that initially infected the individual! One easily understands the difficulties in developing a vaccine...

### ***A real game of hide and seek***

In most viral infections, our immune system manages to eliminate altogether the infectious viral particles after a certain period. This is true in the event of mumps, measles or the flu, for example. Once the infection has been fought off,

we become "seropositive" for the particular virus that infected us. In other words, our system has acquired antibodies that will protect us against a second infection caused by the same virus. But in the case of HIV, our immune system response is insufficient to eliminate all the viruses. Indeed, all over the body there are infected cells in which, as we have seen, the virus remains "hidden". By avoiding detection by the immune system, these cells become "reservoirs" for new viruses and they manage to replicate themselves slowly but progressively in the organism.

Our immune defenses against HIV are ensured by antibodies and the CD8 T lymphocytes. But as we have seen, the CD4 T-lymphocytes act as an orchestra's conductor, directing the B-lymphocytes to produce the antibodies and the CD8 T-lymphocytes to kill the infected cells.

As the number of viruses increases, the number of CD4 T-cells decreases. There are several reasons for this. First when the virus multiplies, the CD4 lymphocytes are destroyed or eliminated by the CD8 T-cells. Secondly, the HIV disturbs communication between cells, so that the CD4 T-cells receive abnormal signals instructing them to either stop replicating or to commit suicide. Finally, the organs that produce new CD4 T-lymphocytes are progressively destroyed once the HIV has invaded them.

#### **Tracking down CAF**

We have known for a long time that the CD8 T-lymphocytes play an important role in the elimination of viruses by killing infected cells. But in 1986, researchers at the University of California in San Francisco, showed that in addition to this, the CD8 T-cells of a number of people infected by HIV-1 virus, secrete a molecule that, *in vitro*, is able to suppress the replication of this virus whatever its type. CAF, for CD8 Antiviral Factor, was the name given to this mysterious molecule. In people who are infected by HIV, but who are healthy from a clinical standpoint, CAF is released by the stimulated CD8 lymphocytes in quantities higher than normal. This is especially true for the "slow progressors". On the other hand, CAF is rarely detected in the CD8 cells of seropositive patients who exhibit obvious signs of immunodeficiency ("progressors"). For the past 16 years, despite using every known method, the true identity of CAF is still unknown. In 1995, a team thought it had hit the jackpot: they discovered that stimulated CD8 cells produce  $\beta$ -chimokines which are able, *in vitro*, to block HIV infections. Unfortunately, it was then discovered that this only worked for viruses that use one of the two types of co-receptor that allow the

invasion of CD4<sup>1</sup>. So unlike CAF, these molecules were incapable of blocking every type of the virus. So, it was back to square one....

#### **But what is CAF?**

In order to answer this question, David Ho of the Aaron Diamond AIDS research Center and his team analyzed and compared samples of CD8 lymphocyte secretions from slow progressors, seropositive progressors and non-infected people who served as the control group. With the aid of powerful protein-chip technology, they discovered that the CD8 lymphocytes of slow progressors secreted, once they were stimulated, a group of 3 proteins, which were absent from all the other samples. Different techniques were then used and these proteins were identified as  $\alpha$ -defensins 1, 2 and 3.

To verify that these three proteins were truly responsible for the anti-HIV activity of CAF, the three proteins were first eliminated from the secretion samples of CD8 cells. Next the anti-HIV activity of the secretion samples was tested on different types of the virus. Although the original secretion samples were able to inhibit 50-60% of the replication of all the viruses that used one of the two co-receptors, once the  $\alpha$ -defensins had been eliminated, the secretion samples basically had no more inhibition activity. These results seemed to indicate that the  $\alpha$ -defensins were responsible for most of the anti-HIV activity of CAF against this type of virus. But for viruses that used the other co-receptor, (those inhibited by the  $\beta$ -chimokines), the decrease in inhibition observed was only 40%. Second test: The anti-HIV activity of synthetic forms of the commercially available  $\alpha$ -defensins 1 and 2, as well as the activity of  $\alpha$ -defensins that were purified from the cells of normal people were tested. The degree of inhibition observed using the commercial products seemed to suggest that these were 10 to 20 times less powerful against HIV than the purified  $\alpha$ -defensins. The researchers explained this fact by noting that the products were probably not pure and that there was no guarantee that the synthetic proteins had the correct shape, which seemed to be important for the activity of  $\alpha$ -defensins. In addition, when tested separately, their anti-HIV activity was rather weak compared to that obtained when the two were combined. This would seem to indicate that the anti-HIV activity of CAF is due to the different  $\alpha$ -defensins interacting together. Finally, by using a fluorescent cell labeling technique, they demonstrated that a small proportion of the CD8 lymphocytes from healthy

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<sup>1</sup> The  $\beta$ -chimokines are hormones that are involved in cell-to-cell communication in the immune system. By binding to the HIV co-receptor, the  $\beta$ -chimokines compete with the virus.

subjects also contained  $\alpha$ -defensins. After stimulation, a small percentage of these cells started to produce some more, thereby confirming that the CD8 lymphocytes are able to produce and secrete  $\alpha$ -defensins. What remains to be done is to identify this particular CD8 lymphocyte subpopulation.

#### ***Not everyone agrees***

But what do we know about these proteins? The defensins are a family of small proteins, which are very abundant in the cells of the immune system. They are known for their antimicrobial activity: acting like a natural antibiotic, they break the bacterial membrane. But a general antiviral activity and more specifically an anti-HIV activity have also been recorded.

Although the results of the New York team are interesting, voices have been raised in several quarters. Several renowned researchers in the field are not convinced and think that the defensins are not the factor that everyone is looking for. For starters, the study is based on too few patients. Secondly, no mechanism is proposed to explain how the defensins block the HIV. Thirdly, it ignores other factors that a number of researchers consider more important. Finally, the observed resistance cannot be explained by such a modest effect.

#### ***While waiting for the vaccine***

Since 1986, researchers from all over the world have been trying to develop a preventive or therapeutic vaccine against HIV but they have encountered many obstacles. Given the numerous failures to date, it is clear that many more years will be needed before a vaccine is available. Meanwhile resistance to tritherapy treatments are starting to appear, so it is clear that much more effort for developing new treatments must be pursued while still continuing to search for a vaccine.

#### ***For further information***

- Zhang L. et al., "Contribution of Human alpha-defensin 1, 2, and 3 to the Anti-HIV-1 Activity of CD8 Antiviral Factor", *Science* 298:995-1000(2002) PMID: 12351674

#### ***On the Internet:***

- Fondation pour la Recherche Médicale (FRM) : [http://www.frm.org/informez/info\\_ressources\\_dossiers\\_article\\_sommaire.php?id=26&type=10&listedossier=26](http://www.frm.org/informez/info_ressources_dossiers_article_sommaire.php?id=26&type=10&listedossier=26)

If, as put forth by the researchers, the activity of the defensins really represents one of the components of CAF, the implications could be important as this would imply that the  $\alpha$ -defensins and the  $\beta$ -chimokines are responsible for reducing the progression of the infection in patients who seem to be favored by specific genetic and environmental factors. If this is true, a better understanding of the production of defensins by the CD8 T-lymphocytes could help to extend its potential benefits to other HIV-positive patients and eventually permit the development of a pharmacological substitute. In addition, by identifying the specific region in these molecules that blocks HIV, it may be possible to increase the efficiency of a synthetic version. However, it may be that defensins are nothing more than non progression markers and are only an indication that, despite HIV infection, the CD8 T-cells are sufficiently stimulated and are still able to produce these small antimicrobial proteins. If so, measuring the amount of the defensins could still be used for determining good "timing" for antiviral therapy. This is why we must follow the defensins' trail wherever it may lead us - even if new studies show that CAF has once more eluded its pursuers...

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***Illustrations:***

- Heading illustration, Source: mature HIV particles, Dr. Hans R. Gelderblom, Robert Koch-Institut, Berlin
- Fig.1, Source: Départements de Biologie du Réseau Collégial Québécois : <http://www.coll-outao.qc.ca/bio/Imagebiologie/Image%20micro.htm>

***At UniProtKB/Swiss-Prot:***

- Defensin 1, Homo sapiens (humain): P59665
- Defensin 3, Homo sapiens (humain): P59666

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