

Summary

Dendritic cells and lymphotropic viruses: Who is fooling whom?

Viral infections

An infection is an inflammation or disease caused by invasion of the body by a pathogen, such as a bacteria, fungus or virus. Everyone is familiar with infections caused by viruses, such as rhinitis, cold sore, flu, rubella, measles and AIDS. These diseases are referred to as viral infections. Viral infections can pass annoying but mildly, such as for a common cold, but can also pass severe and/or never go away, such as for hepatitis B and AIDS.

Lymphotropic viruses replicate in lymphocytes

Viruses are very small particles that we cannot see with our naked eyes. Viruses consist of a small piece of genetic material and some structural proteins that are held together in a cover. Viruses contain some features of life, but they cannot reproduce independently. To create new particles, viruses need a host, such as humans, and within this host viruses need host cells, where they replicate their genetic material, produce new proteins and generate new virus particles.

Viruses often use specific host cells, such as the mucosal cells of our nose or cells of our liver. We have investigated lymphotropic viruses. Lymphotropic viruses have a preference for lymphocytes as their host cells. Lymphocytes comprise an important part of our immune system. We have investigated two lymphotropic viruses: measles virus and human immunodeficiency virus, the causative agent of AIDS.

Viral transmission

Transfer of a virus from one host to another is referred to as viral transmission. On a macroscopic level it is often well understood how the viruses spread, such as via direct body contact (e.g. cold sore), or via blood and sexual contact (e.g. HIV). However, the microscopic events occurring when a virus enters a new host are largely unknown. The virus must hold on to the new host, find itself a way into the body and find its target cells. Lymphotropic viruses must find the lymphocytes, which requires large effort, since lymphocytes are mainly located in deeper parts of the body, such as in blood or lymph nodes. At the site where pathogens invade our body, such as in skin and the mucosa, lymphocytes are very scarce. Thus, an important question remains how lymphotropic viruses manage to find the lymphocytes, without being destroyed by our immune system. Over the past four years, we have studied these processes and our findings are reported in this thesis.

Dendritic cells are the guards of our immune system

As mentioned above, lymphocytes are only scarcely present at the site where pathogens invade our body. A cell type of our immune system that is largely present in these tissues is the dendritic cell (DC). DCs have the function to activate other cells of the immune system, the lymphocytes. In all tissues where pathogens may invade, DCs are located and waiting for action. DCs contain long protrusions (dendrites), which form a network throughout the tissues, ensuring that pathogens will be trapped. On these dendrites the DCs have specialized 'pathogen-detection-sensors'. These sensors, which we call

receptors, are proteins that recognize foreign structures. When a receptor senses such a structure, it will take the pathogen into the DC where it will be degraded. Furthermore, the DC will save small parts of the degraded pathogen to show to the rest of the immune system what is going on at the place of infection. The DC will migrate to the lymph nodes, where the lymphocytes are located. Via signals the DC will inform the lymphocyte where he came from, which pathogens he captured and what he thinks has to happen to respond to the infection. In this way, the lymphocytes can respond with an immune reaction specific to the pathogens.

Dendritic cells: Trojan horse?

While comparing the route of the DC in the immune system with the route a lymphotropic virus aims at to infect the host, we see a parallel. The DCs migrate from the site where pathogens enter our body to the lymph nodes. A lymphotropic virus needs exactly the same route to find its host cells. Around 1990 researchers already described this parallel and suggested that HIV subverts DCs to hitchhike from the place of invasion (the genital mucosal tissues) to places where the lymphocytes are largely present, such as in the lymph nodes. The researchers were able to mimic these events in the lab and reported that this process is likely to play a role in the sexual transmission of HIV. After this report the underlying mechanisms involved in the capture of HIV by DCs and the transmission of HIV from DCs to the lymphocytes have been, and are still being, unraveled.

DCs express the receptor DC-SIGN. It was demonstrated that DC-SIGN is involved in capture of HIV by DCs. In **Section 2** we have demonstrated that DCs express another important receptor for HIV, namely syndecan-3. Syndecan-3 and DC-SIGN are both important for binding HIV to DCs. Unfortunately, the receptors do not degrade the virus, as you would expect from 'proper' pathogen receptors. Instead, these receptors store the virus and allow transmission of the virus from DCs to lymphocytes. These findings could implicate that blocking DC-SIGN and syndecan-3 would be a perfect treatment to stop the HIV epidemic. However, in the next part of this thesis we will make clear that this is not yet evident.

DC-SIGN: universal receptor for viruses?

In **Section 3** and **4** we have investigated whether the mechanisms we described for HIV also hold true for other viruses. First of all, we have explored whether DCs are involved in measles virus infection. We infected monkeys with a green measles virus. The monkeys acquired classical symptoms of measles, but in a green mode: such as green spots in their mouth and on their body. Further analysis of the tissues revealed that the DCs in the monkeys were also green. Thus, measles virus infected DCs, suggesting that DCs play an important role in the dissemination of the virus throughout the body. Subsequently, we investigated whether measles virus, but also other viruses, interact with DC-SIGN. We showed that DC-SIGN is a receptor for a broad range of viruses, including measles virus and herpes simplex virus. We have further investigated the outcome after viruses interacted with DC-SIGN on DCs. For lymphotropic viruses this interaction can result in transmission to lymphocytes, as described before for HIV. However, our results indicate that binding of a virus to DC-SIGN can also result in degradation of the virus and the induction of a viral-specific immune response.

Langerhans cells: Dr. Jekyll and Mr. Hyde?

So far we have considered DCs as one cell type. In fact, DCs are a family of different cell subtypes with roughly, but not exactly, the same function in the immune system. The DC subtype expressing the receptor DC-SIGN (DC-SIGN⁺ DCs) is often located a little deeper in the tissues. However, another subtype, the Langerhans cell (LC), is present in the superficial layers, such as in the upper layer of the skin and mucosa. In intact tissues, this subtype of DCs is the first cell encountering HIV upon transmission. In **Section 5** we have investigated the interaction of HIV and measles virus with LCs. After

the reports on the role of the DC-SIGN⁺ DCs in HIV transmission, we expected a dramatic effect. However, the results turned out completely different than expected. LCs appeared to destroy HIV via a specific receptor, identified as Langerin. The capture and degradation via Langerin was very efficient, cleaning up HIV before it could infect other cells. Also for measles virus we observed a protective role for the receptor Langerin. Moreover, capture by this receptor resulted in the induction of an immune response. Thus, in contrast to DC-SIGN, Langerin does have the expected capacities of a 'proper' receptor.

Our results were somewhat confusing, since other research groups had demonstrated that LCs can be infected with HIV, resulting in transmission to lymphocytes. We found out that transmission of HIV is only possible using high concentrations. We therefore think that the function of Langerin can be saturated. In this situation, LCs change from a cell protecting against HIV into a cell transmitting the virus.

Langerin and DC-SIGN

From **Section 2** and **5** it became evident that the receptors Langerin and DC-SIGN have an opposite role during transmission of HIV. However, the receptors largely resemble each other and belong to the same protein family. From a scientific point of view, it is interesting why these resembling proteins have opposite functions. This might indicate a specific regulation of pathogen capture in LCs and DC-SIGN⁺ DCs. From the point of stopping the HIV pandemic the different functions in combination with the resembling structures is a complicating factor. Blocking DC-SIGN, as we suggested earlier, may be a dangerous idea, since the reagent that interferes with DC-SIGN function might as well block Langerin. To ultimately stop the function of DC-SIGN, specific reagents need to be used, such as the identified compound Lewis X.

Risk factors for HIV

From the work described in the previous sections, it is evident that different processes are involved in the transmission of HIV. Furthermore, different individual characteristics can increase or decrease the chance to acquire HIV. These of course include unsafe sex with different partners, or a partner with a high viral load. But these also include other circumstances, such as having other genital infections or having anal sex. In **Section 6** we investigated whether genital herpes and other bacterial infections affect LC function. We showed that different bacterial structures, but also infection with herpes, change LC phenotype and enhance HIV transmission. We also explored differences between tissues of the vagina, cervix, foreskin and glans penis. We observed that female tissues have less LCs and more lymphocytes compared to male tissues. This might implicate a role for different cell types during sexual transmission of HIV in the two genders.

Novel drugs to inhibit HIV transmission

For certain viral diseases, drugs are available that cure the infection. However, for most viruses we do not have a curative treatment or only drugs that slow down the infection. The best method to protect the population against severe viral diseases is of course prevention of novel infections. This might be done by taking precautions, such as condom use and blood testing or by immunization with an effective vaccine. Unfortunately, a vaccine is not, and might never, be available for every virus. We are therefore continuously looking for new methods to prevent viral infections.

A remaining question is what we can do to prevent transmission of viruses. Our research demonstrated that different factors are involved in transmission. These factors increase or prevent HIV transmission. Influencing these processes might have disastrous consequences. This was recently underlined by a clinical study on a candidate drug to prevent HIV transmission. Instead of preventing transmission, this

drug increased the chance to acquire HIV by a yet unknown reason. In **Section 7** we have examined a novel candidate drug that could be used to prevent transmission of viruses. This compound was effective against infection with HIV and herpes simplex virus. Furthermore, it prevented transmission of HIV via DCs and LCs. This compound is thus a proper candidate, however, further research needs to be done, in order to evaluate whether this compound is effective and does not have side effects.

Conclusions: Who is fooling whom?

The last decennia it became evident that DCs play a crucial role in our immune system. DCs can induce and modulate immune responses. However, different researches including ours have revealed that viruses can influence DC function to survive in a host. The viruses can suppress the viral-specific immune responses or generally suppress the immune system. Furthermore, different experiments suggest that viruses abuse DCs to invade the body. We have demonstrated that DCs can also protect against invading viruses: LCs take up viruses and destroy them. Unfortunately this system is not waterproof and dependent on different factors, such as virus concentrations and LC activation. Since a large part of the research on DCs and virus interactions is performed in the lab, it is the time to translate these results to the 'real life' situation. Future research will further elucidate who is fooling whom, where, when and maybe at one point why...