Annals of Tropical Paediatrics (2001) 21, 191–194

Taking the HIV factory tour

HOWARD STEEL ARMISTEAD, BA, MA, Member of the International AIDS Society

The immune system is the most complex system in the human body. It consists of a number of organs and many types of cell. Immune cells travel throughout the body, protecting us from microbes and viruses in the environment and fighting infection. If the immune system is seriously damaged, as in HIV disease, the body loses its ability to stop opportunistic infectious agents from destroying it.

Cells are the basic building blocks of all living things. The human body is a community of billions of cells that have evolved together and work together for common benefit. Immune cells serve as the army that protects this body of cells from harmful invaders. As in any fighting force, communication within the immune system army is crucial. Cells use chemical messenger signals to communicate between one another and also for communication inside each individual cell. These messenger signals are called cytokines. There are several dozen different cytokines and more are discovered almost every year. This is currently a cutting edge of scientific study in the biological sciences.[1]

Cytokine signals either stimulate or inhibit cellular actions. Nuclear Factor kappa Binding (NF-κB) is a cellular growth factor that plays a critical role in HIV disease. A cellular growth factor is a specialised cytokine that turns on, stimulates and increases growth of cellular protein material after it receives signals from other cytokines to do so. As a cellular growth factor, NF-κB acts as a super-stimulant to HIV production. Understanding how NF-κB works is important in understanding how certain cells manufacture HIV viruses.

Reprint requests to: H. S. Armistead, International Project for Affordable Therapy for HIV, PO Box 691068, Los Angeles 90069, U.S.A.

The CD4 lymphocyte is the primary cell that HIV infects. The easiest way to picture the role NF-κB plays in HIV disease is to think of the CD4 lymphocyte not as a tiny, microscopic cell but as a large industrial manufacturing plant. Human cells are analogous to factories in almost every detail. They even carry their own power-generating apparatus called mitochondria.

Most industrial factories are alike in certain fundamental ways. They have a production line that starts with raw materials which, through a series of manufacturing steps, are transformed into finished products. Like any large industrial plant, the CD4 cell has several different work areas. The four main work rooms are (i) the RNA to DNA transcription area, analogous to the blueprint or model room; (ii) the manufacturing area; (iii) the cutting area; and (iv) the assembly and packaging area.

The initial fusion of the HIV virus to the outside of the cell wall and its incorporation through the cell wall into the cell is similar to the arrival of a guest at a secure industrial facility. The guest is identified by a receptionist (a receptor) and processed through the waiting room, past security and into the plant.

HIV viruses are hundreds of times smaller than the CD4 cells they infect. Viruses do not have the capability to produce proteins. The only way they can reproduce is to infect a cell, commandeer control of its cellular factory and divert the production process from making the proteins needed by the cell to production of viral protein particles to reproduce the virus itself.

The HIV virus infects only a few types of cells. The CD4 lymphocyte, the macrophage and the embryonic macrophage or monocyte

ISSN 0272-4936 print/ISSN 1465-3281/online/01/030191-04 © 2001 The Liverpool School of Tropical Medicine
Carfax Publishing
DOI: 10.1080/02724930120077754

are the primary ones infected, plus a few other specialised cells that have CD4 receptors on their surfaces. CD4 receptors act as the door lock mechanism on these cells, for which HIV carries the key. When HIV collides with one of the immune cells that have CD4 receptors, it attaches to the receptor and its co-receptor. This is the same as putting a key in the keyhole (receptor) and grabbing the doorknob (co-receptor) when entering a room. Once both receptors are attached, the virus fuses with the cell and gradually incorporates itself physically into the cell, infecting it. It is no more difficult for the virus to enter the cell than for a person to enter their workplace through a security reception room.

The reason HIV disease is so devastating is that the CD4 cell controls the activities of all the other types of immune cells. Hence, viral destruction of the CD4 cells is like killing off the generals of an army or destroying the software for your computer system. When the CD4 cells are depleted, the mechanism governing the immune system is lost. This leads to eventual immunological collapse as the body becomes defenceless against viruses, bacteria, fungi, parasites and conditions such as cancers.

Once the retrovirus has entered the CD4 cell, it first needs to change its viral RNA genetic programme into a DNA programme. Human cells produce only DNA, so the RNA programme cannot work on the cell factory's existing production machinery. Thus, translating the RNA programme into a DNA programme is a critical step in HIV replication. This step is similar to changing a computer programme from one format or language for one kind of computer into a format capable of running the same programme on a different computer system. Remarkably, the HIV virus carries an enzyme, *reverse transcriptase*, to accomplish this task. It brings its own copying machine to the CD4 factory gate! Think of the *reverse transcriptase* enzyme as a copying machine that changes the plain RNA copy into a readable DNA factory blueprint. Reverse transcriptase inhibitors (RTIs) that include AZT, DDI, DDC, D4T and 3TC are drugs that interfere with this transcription or copying process by inserting false pieces into the new blueprint and deleting the corresponding original pieces. The DNA copy is a template, a three-dimensional mould. RTIs work by producing a defective master template of the viral programme. When this faulty template arrives on the cellular factory floor, inside the nucleus, proper viral protein copies cannot be made and only defective copies are manufactured. In HIV infection, if the process of reverse transcription is not inhibited, a perfect viral mould will be sent to the cell's nucleus, the HIV factory production line.

Inside the nucleus, the viral model template is used to manufacture long strands of viral protein particles. These long strands of basic viral protein are then sent out of the nucleus to the cytoplasm, to the cutting room and assembly area.

The HIV virus not only carries its own copying machine but also its own cutting tool, the protease enzyme. This enzyme slices the long protein strands into pieces which are assembled into new viral cores and packaged into viral capsules, which are shipped out as new HIV viruses ready to infect other CD4 cells. The now famous protease inhibitors work by jamming the protease enyzme cutting equipment, like sticking a piece of wood between the blades of a pair of scissors. This makes it impossible to assemble new viruses out of the uncut strands of protein particles.[2]

So what part does NF-κB play in the HIV factory?

Nuclear Factor kappa Binding (NF-κB) affects actions inside the cell nucleus. NF-κB growth factor is normally stored throughout the cytoplasm attached to its inhibitor, IkB, which prevents NF-κB from moving into action. NF-κB is attached to I-κB by a lipoprotein bond that restrains NF-κB from entering the cell's nucleus.[3] It has been demonstrated that salicylates, including *aspirin*, help maintain the integrity of this bond between NF-κB and IkB, thus reducing the amount of NF-κB which can enter the nucleus.[4]

Reducing the amount of NF-κB in the

nucleus is important, because NF-κB is the critical transactivating factor for HIV production inside the nucleus.[5,6] This viral production machinery has what could be considered a transmission with three different speed settings. The more NF-κB available inside the nucleus, the faster the viral protein particle strands are produced. The name of this controlling switch located at the end of the HIV viral template is the long terminal repeat (LTR) section of the virus.[7,8]

NF-κB can be compared with the factory foreman. Before the NF-κB foreman arrives, the workers are at ease, doing a little work but not accomplishing much and certainly not working fast or very hard. When the foreman arrives, however, it is full speed ahead! When sufficient NF-κB is present, the factory goes on a wartime footing, working overtime. This happens when an infection occurs and the CD4 cell has to manufacture a lot of new protein to create new CD4 cells required for an adequate immune response to the infecting agent. But once HIV has invaded the CD4 cell, it no longer produces CD4 cell proteins but makes HIV viral protein particles instead.

The HIV viruses' LTR switch contains two receptors for the NF-κB protein. If no NF-κB enters the nucleus, then viral production proceeds very slowly. If one molecule of NF-κB attaches to one of its two LTR receptors, this increases the manufacturing pace to a moderate rate. If a second NF-κB molecule docks at the other receptor at the LTR end of the virus, viral production goes into overdrive at very high speed. Thus, the more NF-κB there is in the nucleus, the more HIV component protein will be produced. Both laboratory and clinical trials have shown that aspirin-like drugs can reduce HIV production by about 60%.[4,9] Since NF-κB is a required human cellular molecule, there is always an essential minimum level of NF-κB in the nucleus. Thus, we cannot expect aspirin to reduce NF-κB below that essential normal level; it cannot completely eliminate NF-κB in the nucleus.

A number of other chemical compounds inhibit NF-κB, but most have significant negative side-effects. A mineral supplement that is safe and effective in reducing NF-κB is the anti-oxidant *selenium*. Of all the vitamins and minerals tested against HIV, *selenium* is the only one that has shown a statistically significant ability to extend the life of people with HIV.[10] Selenium should be taken in doses of 50–200 mcg per day.[11] Selenium poisoning occurs when daily doses exceed 2,500 mcg.

Both aspirin and selenium are partially effective against HIV by targeting one of the several cellular processes required to produce new viruses. By itself, aspirin can reduce HIV production by about 60%, which is approximately as effective as AZT against HIV. While this is not nearly as effective as the newest generation of anti-HIV drugs, it is still quite significant.

A 60% reduction in HIV is an approximately half 'log' drop in viral load. Dr John Mellors, a noted authority and head of AIDS research at the University of Pittsburgh, has stated that any drug which results in a half-log reduction in HIV viral load will extend the life-span of those living with HIV (personal communication).

The importance of aspirin and selenium is not only that they are partially effective against HIV but also that they work by a completely different mechanism from the eight reverse transcriptase inhibitors and seven protease inhibitors currently available to Americans with HIV.

Whether in an industrial plant or in a human cell, factory processes have a multiplier effect. The efficiency or lack of efficiency of one production process times that of the next production process, times that of the next, yields the total factory efficiency or total factory output. Multiplying the ability of one drug to inhibit a particular process times the ability of another drug to inhibit another process gives the total ability to inhibit viral reproduction. Logically, inhibiting the cell's factory production process at three different stages gives a better overall result than inhibiting viral replication at only two different points in the production process. Aspirin and selenium are effective anti-retroviral agents whose mechanism of action in reducing viral growth is differ-

ent from that targeted by RTIs and PIs. Using these NF-κB inhibitors along with reverse transcriptase and protease inhibitors must be better than using RTIs and PIs alone. NF-κB inhibitors add a third dimension to HIV combination therapy. For most people in Africa, Asia and Latin America NF-κB inhibitors offer available, effective, affordable treatment against HIV.

It is simple logic to conclude that adding the NF-κB inhibitors, aspirin and selenium, to RTIs and PIs in the fight against AIDS will improve the prognosis, even in affluent societies. The critical question for those living with HIV is how long will it take those who determine AIDS research priorities and funding to comprehend the importance of NF-κB inhibitors in fighting HIV. Safe, cheap and effective NF-κB inhibitors such as aspirin and selenium, as well as other drugs that work by this mechanism, urgently require further testing in order to bring treatment to the millions who have so far been economically denied access to antiviral therapy.

References

1 Mantovani A, Dinarello CA, Ghezzi P. Pharmacology of Cytokines. New York: Oxford University Press, 2000.
2 Levy JA. HIV and the Pathogenesis of AIDS, 2nd edn. American Society of Microbiology Press, 1998.
3 Beauparlant P, Hiscott J. Biological and biological inhibitors of the NF-kB/Rel proteins and cytokine synthesis. Cytokine Growth Fac Rev 1996; 7:175–90.
4 Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 1994; 265:956–9.
5 Alcami J, Lain de Lera T, Folgueira L, *et al.* Absolute dependence on kappa B responsive elements for initiation and Tat-mediated amplification of HIV transcription in blood CD4 T lymphocytes. EMBO J 1995; 14:1552–60.
6 Jacque JM, Fernandez B, Arenzana-Seisdedos F, *et al.* Permanent occupancy of the human immunodeficiency virus type 1 enhancer by NF-kappa B is needed for persistent viral replication in monocytes. J Virol 1996; 70:2930–8.
7 Kopp EB, Ghosh S. NF-kappa B and rel proteins in innate immunity. Adv Immunol 1995; 58:1–27.
8 Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D, Miyamoto S. Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. Genes Dev 1995; 9:2723–35.
9 Kotler DP, Reka S, Hecker L, Cohen S, Clayton F, Tierney AR. Treatment of HIV-associated mucosal inflammation with oral 5-ASA. Gastroenterology 1992; 102:648A.
10 Baum MK, Shor-Posner G, Lai S, *et al.* High risk of HIV-related mortality is associated with selenium deficiency. J Acq Immun Defic Syndr Hum Retrovir 1997; 15:370–4.
11 Goodman & Gilman. The Pharmacological Basis of Therapeutics, 8th edn. New York: Pergamon, 1990.