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March 1991

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An Autoimmune Mechanism for AIDS' T4 Lymphopenia

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Abstract.
We put forward a new model for the T4 lymphopenia occurring in AIDS by suggesting a mechanism whose net effect is blocking the generation of T4 cells during HIV infection.

The scientific community has responded most impressively to the challenge of AIDS. By now, we understand many of its mechanics in great detail. Let us summarize the emerging picture with a few, large strokes. The etiological agent of AIDS has been identified in the HIV virus. More precisely, HIV is a retrovirus with very long latency, whose action is directed against a subpopulation of T cells. T cells, a fundamental component of our immune system, can be divided in two subpopulations: T4 cells and T8 cells. The first is characterized by the expression of glycoprotein CD4 as a surface receptor, the second by glycoprotein CD8; both subpopulations are essential for properly mounting an immune response. HIV does not affect T8 cells, but infects and ultimately causes the death of T4 cells by specifically binding to their CD4 receptor. Though the immune system mounts a vigorous humoral response to HIV, within a few years AIDS patients progressively develop a characteristic T4 lymphopenia. Once T4 cells become sufficiently few, the immune system no longer works properly, AIDS patients fall prey to all kinds of opportunistic infections, and eventually die.

So summarized, the above clinical picture appears tragically clear. However, a mysterious aspect --not highlighted in our short summary-- still exists.

A mystery. HIV infects and ultimately kills T4 cells by binding to CD4. It is thus quite natural to attribute the slowly developing T4 lymphopenia of AIDS patients to the selective depleting action of HIV. But things are not that

simple: the number of T4 cells infected by HIV is *negligible* with respect to the greater number of "missing" T4 cells.

Indeed, Gallo and Montagnier [GM] do not think that HIV's direct killing of T4 cells is sufficient for explaining the depletion seen in AIDS, and ask which indirect mechanisms may also be at work. We wish to suggest such a mechanism.

An Indirect Mechanism

Receptors CD4 and CD8 play, respectively, a major role in the activation of T4 and T8 cells (review by Bierer, Sleckman, Ratnofsky, Burakoff [BSRB]). Indeed, T4 cells recognize antigen in the context of MHC class II proteins (ligands for CD4), and T8 cells in the context of MHC class I proteins (ligands for CD8). An equally important role is played by CD4 and CD8 during thymic development. As shown by Ramsdell and Fawlkes [RF], their engagement is *required* for the maturation of, respectively, T4 and T8 cells. Indeed, it is in the thymus that maturing T cells are selected on the basis of their capability of recognizing antigen. We now ask the following question:

What will happen if the thymus is injected with soluble CD4?

Our answer is that *the maturation of T4 cells will be inhibited.* This appears quite reasonable. In the experiments of Ramsdell and Fawlkes, the role of CD4 in T4-cell maturation was established by injecting anti-CD4 antibodies that do not deplete periphery T4 cells nor double positive thymocytes. Simply *blocking* --with a proper mAb-- the CD4 receptors of maturing T4 cells also blocked their development. We believe that the main effect of this blocking consisted of preventing the interaction of CD4 with thymic class II MHC molecules, and that in turn this caused the blocking of development. However, this interaction may be prevented in a different, but symmetric manner, namely, by blocking thymic class II MHC proteins, something that may be accomplished by soluble CD4.* At this point, a second question naturally arises. Namely,

Is soluble CD4 ever injected in the thymus?

We now argue that this event may indeed be an indirect result of HIV infection:

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* An indirect confirmation that blocking class I (II) MHC molecules may block the development of maturing T4 (T8) lymphocytes, may be inferred from the results of Zijlstra, Bix, Simister, Loring, Raulet, and Jaenish [ZBSLRJ]. They show that genetically engineered mice not expressing class I MHC proteins (not in the thymus nor anywhere else) lack $CD4^{-}8^{+}$ cytolytic cells.

> As for all foreign organisms, our body must react to HIV producing antibodies to all of its antigenic determinants. One of these determinants is known to be a binding site for CD4. Thus we hypothesize that one of the antibodies raised against HIV must have a binding affinity *very close* to the one of CD4. Let's call *CD4copy* such an antibody. Like the copy of a key, *CD4copy* may be vastly different from CD4, but will essentially have its functional value. Thus, it is capable of binding to MHC-II molecules of thymic cells, preventing the development of maturing T4 cells.

Let us further elucidate this basic mechanism. The release in the blood of *CD4copy* caused by HIV will not be a one-time affair. HIV has a very long latency, during which it is "invisible" to the immune system. In these conditions *any* virus would remain present for a long time. (This would hold even if the infected cells were not T4 ones and the immune system had mastered the *instant* killing of HIV whenever it sees it. In fact, provided that sufficiently many cells were initially infected, there would never be a time in which all viruses will become simultaneously visible --either by being free in the blood, or "protruding" from infected T4 cell surfaces.) Thus HIV "continuously reappears," each time eliciting powerful secondary responses of the immune system. Consequently with our hypothesis, (different) plasma cells will continually produce (different) antibodies against HIV, *including CD4copy*. In fact, despite its constant mutability, HIV maintains its capability of binding CD4; thus, once a *CD4copy* antibody has been successfully "manufactured," its continual production will be guaranteed by the *memory* of the immune system, by the *continual reappearance* of HIV, and by the presence on the virus' surface of an *identical* binding site for CD4.

An important, novel feature of our hypothesized mechanism is that it provides a better model for that T4-cell depletion that is the hallmark of AIDS; a model, that is, that explains the mentioned mystery away. In fact, HIV *does not need* to directly kill lots of T4 cells to cause AIDS' impressive T4 lymphopenia. (In principle, it might not need to directly kill a single T4 cell!) It would be sufficient for it to be "visible" for a long time to the immune system, so as to elicit for a long time the production of *CD4copy*, and thus misleading our organism into producing less T4 cells. These cells, like all others, naturally die and need to be replaced; tampering with their replacement may be HIV's most insidious action. In a few years time, it may easily cause the typical T4 lymphopenia of AIDS patients, even without any direct killing. If HIV only caused a modest, selective depletion of easily replaceable T4 cells, it is conceivable that AIDS patients might adjust to living with it.

The emerging etiology for AIDS' T4 lymphopenia is thus that of an autoimmune mechanism. This is in agreement with Giorgi's and Dentels' [GD] remark that T4 depletion occurs only after antibody formation against HIV, though the

presence of antigen can be documented prior to seroconversion. The emerging picture is also easily reconcilable with the fact that the body produces a vigorous response to HIV, as it is exactly this powerful response that causes T4 cell loss.

Testing The Mechanism

Rather than testing directly the inhibition of T4-cell development by *CD4copy*, it may be preferable to perform indirect experiments first.

A succedaneous test may consist of monitoring T-cell reconstitution of irradiated animals, both in the presence and in the absence of soluble CD4. Radiation will cause a sudden drop in T-cell level, and thus shorten the length of the experiment by an accelerated generation of new T cells. Working with soluble CD4 avoids isolating *CD4copy* among many candidate antibodies. Moreover, a new successful method for producing soluble rat CD4 has recently been obtained by Davis, Ward, Puklavec, Willis, Williams, and Barkley [DWPWWB]. However, several experimental challenges remain even in this domesticated scenario. Soluble CD4 lasts in circulation much less than an antibody, so a method must be found to keep a sufficiently high level of it in the blood (or perhaps solely in the thymus) and the right quantity of soluble CD4 must be determined so that its effects --if any-- could be quickly observed. It is also important to notice that, due to the difference in molecular weight and structure between *CD4copy* and soluble CD4, the risk exists that only the former protein may successfully block thymic MHC-II cells.

Using thymocultures and bone-marrow cells may be used to verify that the thymus of AIDS patients indeed produces fewer T4 cells than normal. Among other difficulties, this approach would involve focusing solely on the newly formed T cells. (One solution to this latter problem may be continually but slightly irradiate the thymic tissue, without harming the thymic tissue. The amount of radiation should be such to kill only those lymphocytes that have been exposed to it for much longer than the time needed for T-cell maturation. If this procedure is kept up while precursor cells are continually added, after a while the only lymphocytes present will be newly matured ones.)

An elegant and simpler method has been suggested by Herman Eisen. If *CD4copy* successfully blocks MHC-II molecules in the thymus, the same should happen for these molecules in B cells. Thus, we expect that some binding sites of the constant regions of MHC proteins of AIDS patients will no longer be available, since they are occupied by *CD4copy*. This prediction can be tested by showing that some (fluorescent) antibodies for non-polymorphic MHC-II molecules fail to bind to B cells of AIDS patients. The test can be improved by

using both antibodies (labelled "green") for the polymorphic regions of MHC-II proteins, and (labelled "red") for the constant regions, and then studying the green/red binding ratio for B cells of AIDS patients and of healthy individuals.

In Sum

Above we have described the most plausible way, in the light of established biological mechanisms, for *CD4copy* to influence the level of T4 cells. So little, however, is known about T-cell regulation, that many other possibilities exist for *CD4copy* to affect the level of T4 cells, even in mature adults whose thymus may be atrophic. (For instance, an anti-antibody may be raised against *CD4copy* capable of binding CD4 and depleting T4 cells. For all we know, it may even be that the level of T4 cells is controlled by the *total* amount of CD4 --whether or not on cell surface-- thus allowing *CD4copy* to mislead our organism into believing that there are many more T4 cells than actually present.) For this reason let us summarize our autoimmune model for AIDS' T4 lymphopenia in a more open-ended manner. Namely,

CD4copy causes loss of T4 cells.

Models have a fundamental role in organizing our thoughts and pointing out new possibilities for deeper understanding, but, of course, the last word always belongs to Experiment.

Aknowledgements

We wholeheartedly thank Leonard Adleman for his unique introduction to the problematics of AIDS, and Nancy Hopkins and Herman Eisen for very insightful, very enjoyable, and *very endless* discussions of these ideas and the fascinating field of immunology at large.

A special thanks to Cecil Green for encouraging me to investigate a new field. I am enjoying it tremendously.

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